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Identification of a pubescence locus in Chinese flowering cabbage (*Brassica rapa* L.)

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Abstract

The pubescence is an important trait for *Brassica rapa* vegetables concerning insect resistance but causes a bad mouthfeel when eaten. Pubescence is observed in some Chinese flowering cabbage (*B. rapa*) cultivars. The cleaved amplified polymorphic sequence (CAPS) markers linked to pubescence-related genes *BrTTG1* and *BrGL1* were investigated in a segregating population. The correlation between the presence/absence of pubescence and DNA marker genotypes tested using the Mann-Whitney *U* test, showing that the pubescence trait was significantly correlated with the CAPS marker linked to *BrGL1*. Sequence comparison of *BrGL1* showed several nucleotide polymorphisms between parental cultivars, of which 2-bp deletion caused a frame-shift mutation in the *BrGL1* of hairless cultivar 'Hanakazari'. A CAPS marker developed from *BrGL1* sequences co-segregated with the pubescence trait in this population. Therefore, it suggested that the *BrGL1* is a likely candidate gene controlling the pubescence trait in this Chinese flowering cabbage line.

Keywords: *Brassica rapa, CAPS marker, Chinese flowering cabbage, GL1, pubescence*

Introduction

Chinese flowering cabbage is a vegetable in *Brassica* species, called "*nabana*" or "*hanana*" in Japanese, and is a spring vegetable. It is an associated heirloom vegetable in Kyoto, Japan, which is thought to be derived from oilseed *B. rapa* landraces. This crop is harvested at bolting stage for the buds, stems, and young leaves, such as broccoli and cauliflower in *B. oleracea*. This vegetable is indispensable for the Japanese table due to its vivid leaf color and abundant nutrients such as vitamin C and iron (Ishida, 2004). Therefore, we performed their disease management and classification to promote production (Sunada *et al*., 2018; Kubo *et al*., 2019a; Tsuji *et al*., 2019, 2021).

Some *Brassica* crops possess pubescence (trichome and hair), a hair-like structure observed in the epidermis. Pubescence presents in many plants, that involved in the protection from herbivorous insects and pathogenic microorganisms, resistance to freezing and UV radiation, and temperature and water control (Wagner *et al*., 2004; Kim *et al.*, 2011). Though, it plays a vital role in plant protection, hairy leaves and stems cause bad mouthfeel when eaten. It is important to understand the genes involved in pubescence in breeding programs based on these conflicting aspects. Furthermore, several genes related to pubescence have been reported in *B. rapa*. The *BrTTG1*, an ortholog of *TRANSPARENT TESTA GLABRA 1* (*TTG1*) gene encoding a WD40 repeat protein in *Arabidopsis thaliana*, functions in trichome formation in *B. rapa* (Zhang *et al*., 2009). Also, *BrGL1* is a homolog of the *Arabidopsis GLABRA1* (*GL1*) gene and is a MYB transcription factor for trichome development (Li *et al*., 2009; Kawakatsu *et al*., 2017). We previously detected polymorphism of *BrTTG1* in the F_2 population derived from a cross between Chinese cabbage and turnip lines. A simple sequence repeat marker, BRMS-014, linked to the *BrGL1* gene, was located within a chromosomal region for pubescence trait on the linkage group A6 (Kubo *et al*., 2010). In contrast, it is unknown whether these genes work in Chinese flowering cabbage cultivars. Therefore, we used a Chinese flowering cabbage line segregating the pubescence trait in this study, which was developed during our breeding programs of Chinese flowering cabbage cultivars, to investigate the pubescence gene markers.

Materials and Methods

Two Chinese flowering cabbage (*B. rapa*) cultivars were used in this study: hairy 'CR Kyobare' (Marutane Seed Co., Ltd., Kyoto, Japan) and hairless 'Hanakazari' (Sakata Seed Corporation, Yokohama, Japan). Hairy F_2 individual derived from a cross between the two cultivars

backcrossed with 'Hanakazari' as a recurrent parent to develop a backcrossed (F_2BC_1) population segregating the presence/absence of pubescence. Two subsets of F_2BC_1 seeds (n = 114 and 20) with the two parental cultivars (n = 20 each) were sown in pots and grown in a greenhouse. The former F_2BC_1 subset grown at 25^0 C under a long day condition (16 h light/8 h dark) for two weeks. The latter subset transplanted after two weeks (early October, 2020) to an open field of the University Farm, and grown under natural conditions for 2.5 months (Tsuji *et al*., 2019). Presence/absence of the pubescence inspected visually and scored into two classes (0: absence, 1: presence) at the development of true leaves for the former subset and in mid-December 2020 for the latter subset. Genomic DNA extracted from a fresh leaf of the former subset's individuals as previously reported (Kubo *et al*. 2019b). The entire coding regions of *BrGL1* in 'CR Kyobare' and 'Hanakazari' were amplified with a primer pair (forward: 5'-ATGAGAACGAGGA GAAGAACA-3' and reverse: 5'-CTAGAGGCAGTAG CCAGTATCA-3'), cloned into a pCR-4-TOPO vector (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), and sequenced (their nucleotide sequences have been deposited to the DDBJ/ENA/GenBank databases under the accession nos. LC669916-LC669918). PCR reaction of three cleaved amplified polymorphic sequence (CAPS) markers, *BrTTG1*/*Apa*I, KBrS004A14s/*Hha*I, and *BrGL1*/*Nde*I, were performed according to the previous reports (Li *et al*., 2009; Zhang *et al*., 2009; Kubo *et al*., 2010), in which the KBrS004A14s/*Hha*I CAPS had been developed from the BAC clone KBrS004A14 containing the *BrGL1* gene (accession no. AC189633.2) (Kubo *et al*., 2010), 74.4 kb apart from the *BrGL1* gene. PCR products were digested with *Apa*I, *Hha*I, and *Nde*I, respectively, followed by electrophoresis in 1.5% or 3% agarose gel. The significance between the two groups (absence and presence of pubescence) and the genotypes of each marker tested by the Mann-Whitney *U* test with BellCurve for Excel 3.21 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results and Discussion

Two weeks after sowing, 57 hairy and 57 hairless individuals were observed in the former subset of the F_2BC_1 population derived from a backcross of an F_2 plant (hairless 'Hanakazari' × hairy 'CR Kyobare'). Nine and 11 F_2BC_1 individuals had hair and no hair in the latter subset, respectively. Each 20 individuals of the two parental cultivars ('CR Kyobare' and 'Hanakazari') were hairy and hairless, respectively (data not shown). These results strongly suggest that the pubescence trait is controlled by a single locus in this population. We tested two existing DNA markers, *BrTTG1*/*Apa*I and KBrS004A14s/*Hha*I CAPSs, for pubescence-related genes, *BrTTG1* and *BrGL1*, respectively. In the F_2BC_1 population, *BrTTG1*/*Apa*I CAPS was segregated into three genotypes (A: complete digestion, B: no digestion, and H: heterozygote between A and B), whereas KBrS004A14s/*Hha*I CAPS showed the two genotypes (A and H). The genotypes correlated with the pubescence trait in the KBrS004A14s/*Hha*I marker except for one individual, but not in the *BrTTG1*/*Apa*I marker (Fig. 1, center and left panels).

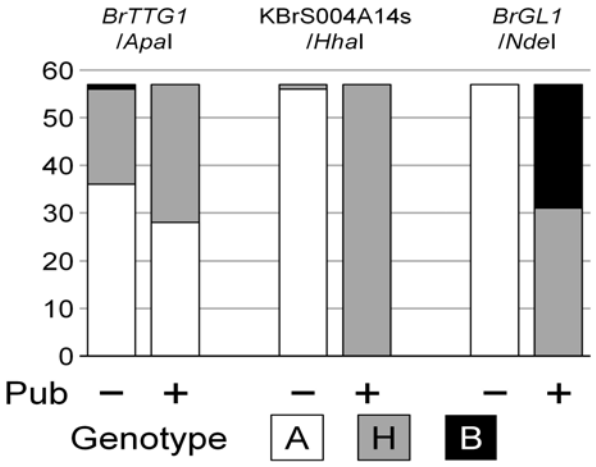

Fig. 1: Frequency distributions of the F_2BC_1 population regarding the pubescence trait (Pub) and genotypes of three CAPS markers. -, +: absence and presence of pubescence, respectively. Box: genotypes of *BrTTG1*/*Apa*I, KBrS004A14s/*HhaI*, and *BrGL*1/*Nde*I CAPS markers (A: complete digestion, B: no digestion, and H: heterozygote between A and B). Individual number indicated on the left side

The Mann-Whitney *U* test between the presence and absence of pubescence showed p = 0.076 in *BrTTG1*/*Apa*I and p<0.001 in KBrS004A14s/*Hha*I (Table 1). The null hypothesis in the KBrS004A14s/*Hha*I marker rejectable at the 1% level, indicating a significant correlation between the pubescence trait and the KBrS004A14s/*Hha*I (*BrGL1*-linked marker) in this population. Therefore, we sequenced the *BrGL1* gene in the two parental cultivars ('CR Kyobare' and 'Hanakazari') of the population. These two cultivars had two and one alleles, respectively, of which one of the two alleles (allele 1) in 'CR Kyobare' transmitted to the F_2BC_1 population. Schematic representation of *BrGL1* gene is shown in Fig. 2, upper panel (filled box: protein coding region, gray box: coding region truncated by a 2-bp deletion, horizontal line: intron). Their partial nucleotide sequences are also indicated in Fig. 2, lower panel (upper case letters: exon 3,

Table 1: Results of the Mann-Whitney *U* test in the F_2BC_1 population

Marker	Pub	Genotype			Total	*U* value	*z* value	*p* value
		A	H	B				
BrTTG1/*Apa*I	0	36	20	1	57	1354	1.78	0.076
	1	28	29	0	57			
KBrS004A14s/*Hha*I	0	56	1	-	57	28.5	10.44	<0.001**
	1	0	57	-	57			
BrGL1/*Nde*I	0	57	0	0	57	0	10.03	<0.001**
	1	0	31	26	57			

Pub: pubescence phenotype (0: absence, 1: presence)
Genotype of each CAPS marker (A: complete digestion, B: no digestion, and H: heterozygote between A and B)
U value: the smaller *U* value of the two samples
z value: approximated to the standard normal distribution
**: significant at 1% level

lower case letters: 3'-acceptor site of the intron 2). The sequence comparison showed several nucleotide polymorphisms between the parental cultivars, of which a 2-bp deletion (Fig. 2, lower panel, highlight) caused a frame-shift mutation in the *BrGL1* of a hairless cultivar 'Hanakazari' (Fig. 2, lower panel, italics). Also, a CAPS marker, *BrGL1*/*Nde*I, which was developed from a polymorphism at the *Nde*I site (Fig. 2, upper panel, arrowhead), co-segregated with the pubescence trait in this population (Fig. 1, right panel, Table 1). The 'CR Kyobare' homozygous (B) genotype appeared in 26 individuals with *BrGL1*/*Nde*I CAPS (Fig. 1, right panel, Table 1), which was unexpected in the BC_1 generation. We have no idea for the reason of such genotype, although there was a complete correlation between the hairless phenotype and the 'Hanakazari' homozygous (A) genotype.

It has been reported that pubescence trait is controlled by a dominant locus in *B. rapa* in many cases (Song *et al.,* 1995; Lou *et al*., 2007; Kubo *et al.*, 2010; Kawakatsu *et al*., 2017). In this study, we investigated the correlation

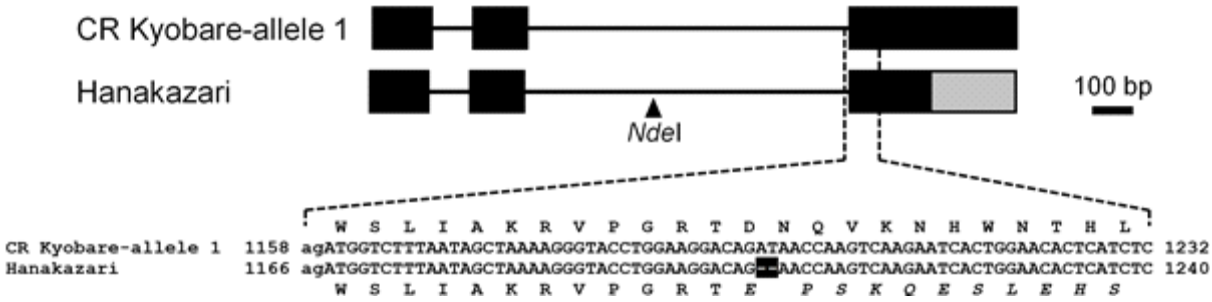

Fig. 2: Comparison of *BrGL1* gene in hairy 'CR Kyobare' (allele 1) and hairless 'Hanakazari'. In lower panel, nucleotide positions are indicated at the left and right of the nucleotide sequences. Predicted amino acid sequences in 'CR Kyobare' (allele 1) and 'Hanakazari' are indicated above and below nucleotide sequences, respectively

between the pubescence trait and the genotypes of three DNA markers (*BrTTG1*/*Apa*I, KBrS004A14s/*Hha*I, and *BrGL1*/*Nde*I CAPSs) in Chinese flowering cabbage. The *BrGL1* marker was significantly correlated with the pubescence trait but the *BrTTG1* marker was not (Fig. 1). This result agreed with the segregation of the pubescence trait (57/57 and 9/11 of hairy/hairless individuals in the two subsets), which fitted to the 1:1 expected ratio in the BC_1 generation. These results suggest that the *BrGL1* gene is a likely candidate gene controlling the pubescence trait in this population. Furthermore, polymorphism was detectable in the *BrGL1* gene, and its polymorphism was detected using a restriction enzyme, *Nde*I, in the present Chinese flowering cabbage lines. There were mutations in the hairless cultivar, truncating the coding region of *BrGL1* (Fig. 2). Deletions in the coding regions of *BrGL1* homolog and *BrTTG1* resulted in non-functional proteins (Zhang *et al*., 2009; Kawakatsu *et al*., 2017). Additional study to determine the correlation between *BrGL1* polymorphism and pubescence trait in other *B. rapa*

vegetables, to examine the expression of *BrGL1* alleles, and to understand the mechanisms of pubescence formation may also be helpful for future breeding of *Brassica* vegetables.

Conclusion

The genotypes of the F_2BC_1 population in Chinese flowering cabbage were investigated using three DNA markers (*BrTTG1*/*Apa*I, KBrS004A14s/*Hha*I, and *BrGL1*/*Nde*I CAPSs). When the correlation between the pubescence trait and genotypes of the three markers was tested, the *BrGL1*/*Nde*I CAPS correlated with the pubescence trait. This CAPS marker would be useful for further breeding of Chinese flowering cabbage.

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References

Ishida M. 2004. Tsukena-rui. In: Outline of Agricultural Technology, Vegetables Vol. 7. Rural Culture Association Japan (ed) Tokyo, Japan, Rural Culture Association Japan, pp 5-7.

Kawakatsu Y, Nakayama H, Kaminoyama K, Igarashi K, Yasugi K, Kudoh H, Nagano AJ, Yano K, Kubo N and Kimura S. 2017. A *GLABRA1* ortholog on LG A9 controls trichome number in the Japanese leafy vegetables Mizuna and Mibuna (*B. rapa* L. H. Bailey): evidence from QTL analysis. *J Plant Res* **130**: 539-550.

Kim HJ, Han JH, Kim S, Lee HR, Shin JS, Kim JH, Cho J, Kim YH, Lee HJ, Kim BD and Choi D. 2011. Trichome density of main stem is tightly linked to PepMoV resistance in chili pepper (*C. annuum* L.). *Theor Appl Genet* **122**: 1051-1058.

Kubo N, Saito M, Tsukazaki H, Kondo T, Matsumoto S and Hirai M. 2010. Detection of quantitative trait loci controlling morphological traits in *Brassica rapa* L. *Breed Sci* **60**: 164-171.

Kubo N, Onnazaka K, Mizuno S and Tsuji G. 2019a. Classification of "nabana" (*B. rapa*) cultivars and landraces based on simple sequence repeat markers. *Breed Sci* **69**: 179-185.

Kubo N, Ueoka H and Satoh S. 2019b. Genetic relationships of heirloom turnip (*B. rapa*) cultivars in Shiga Prefecture and other regions of Japan. *Hort J* **88**: 471-480.

Li F, Kitashiba H, Inaba K and Nishio T. 2009. A *B. rapa* linkage map of EST-based SNP markers for identification of candidate genes controlling flowering time and leaf morphological traits. *DNA Res* **16**: 311-323.

Lou P, Zhao J, Kim JS, Shen S, Carpio DPD, Song X, Jin M, Vreugdenhil D, Wang X, Koornneef M and Bonnema G. 2007. Quantitative trait loci for flowering time and morphological traits in multiple population of *B. rapa*. *J Exp Bot* **58**: 4005-4016.

Song K, Slocum NK and Osborn TC. 1995. Molecular marker analysis of genes controlling morphological variation in *B. rapa* (syn. *campestris*). *Theor Appl Genet* **90**: 1-10.

Sunada K, Ono U, Kimura S, Kubo N and Tsuji G. 2018. Assessment of various plants to reduce the population density of *Plasmodiophora brassicae* in soil. *Ann Rept Kansai Pl Prot* **60**: 81-84.

Tsuji G, Kamibayashi Y, Hotta Y, Ono U, Kimura S, Date S and Kubo N. 2019. Impact of clubroot disease on the yield of Hanana (flower nabana). *Ann Rept Kansai Pl Prot* **61**: 129-132.

Tsuji G, Date S, Tanaka S, Ono U, Kimura S and Kubo N. 2021. Control of clubroot disease on Hanana (flower nabana) using dry seaweed powders. *Ann Rept Kansai Pl Prot* **63**: 13-20.

Wagner GJ, Wang E and Shepherd RW. 2004. New approaches for studying and exploiting an old protuberance, the plant trichome. *Ann Bot* **93**: 3-11.

Zhang J, Lu Y, Yuan Y, Zhang X, Geng J, Chen Y, Cloutier S, McVetty PBE and Li G. 2009. Map-based cloning and characterization of a gene controlling hairiness and seed coat color traits in *B. rapa*. *Plant Mol Biol* **69**: 553-563.