Theoretical and Applied Genetics (2021) 134:701–714
https://doi.org/10.1007/s00122-020-03724-8

ORIGINAL ARTICLE

Fine mapping of QTL conferring Cercospora leaf spot disease resistance in mungbean revealed TAF5 as candidate gene for the resistance

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Received: 24 May 2020 / Accepted: 3 November 2020 / Published online: 13 November 2020

Abstract

Key message **This paper reports fine mapping of *qCLS* for resistance to Cercospora leaf spot disease in mungbean and identified *LOC106765332*encoding TATA-binding-protein-associated factor 5 (TAF5) as the candidate gene for the resistance**

Abstract Cercospora leaf spot (CLS) caused by the fungus *Cercospora canescens* is an important disease of mungbean. A QTL mapping using mungbean F_2 and BC_1F_1 populations developed from the "V4718" (resistant) and "Kamphaeng Saen 1" (KPS1; susceptible) has identified a major QTL controlling CLS resistance (*qCLS*). In this study, we finely mapped the *qCLS* and identified candidate genes at this locus. A BC_8F_2 [KPS1 × (KPS1 × V4718)] population developed in this study and the F_2 (KPS1 × V4718) population used in a previous study were genotyped with 16 newly developed SSR markers. QTL analysis in the BC_8F_2 and F_2 populations consistently showed that the *qCLS* was mapped to a genomic region of ~ 13 Kb on chromosome 6, which contains only one annotated gene, *LOC106765332* (designated "*VrTAF5*"), encoding TATA-binding-protein-associated factor 5 (TAF5), a subunit of transcription initiation factor IID and Spt-Ada-Gcn5 acetyltransferase complexes. Sequence comparison of *VrTAF5* between KPS1 and V4718 revealed many single nucleotide polymorphisms (SNPs) and inserts/deletions (InDels) in which eight SNPs presented in eight different exons, and an SNP (G4,932C) residing in exon 8 causes amino acid change (S250T) in V4718. An InDel marker was developed to detect a 24-bp InDel polymorphism in *VrTAF5* between KPS1 and V4718. Analysis by RT-qPCR showed that expression levels of *VrTAF5* in KPS1 and V4718 were not statistically different. These results indicated that mutation in *VrTAF5* causing an amino acid change in the VrTAF5 protein is responsible for CLS resistance in V4718.

Communicated by Henry T. Nguyen.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00122-020-03724-8) contains supplementary material, which is available to authorized users.

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Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is an important crop in Asia (Chankaew et al. 2011). It is widely grown in South, East, and Southeast Asia, especially in India, Myanmar, and China. It is also grown as a cash crop in Australia, Canada, and some African countries. The total cultivation area of this crop is about 6–7 million hectares (Nair et al. 2012). Due to its short life cycle of about 60–70 days and relatively robust drought tolerance, mungbean is mainly cultivated as a component in various agricultural systems (Chankaew et al. 2011). In Asia, mungbean is mainly cultivated before and after rice, maize, and wheat. It is also intercropped with cotton and sugar cane. Mungbean seeds are consumed in a variety of ways and processed into several products, including sprouts, noodles, starch, alcohol, and cosmetics.

The average seed yield of mungbean cultivated in Asia is low, being about less than 1 ton per hectare (Nair et al.

2012). One of the main factors causing the low yield is disease. Major and common diseases in mungbean production in Asia include powdery mildew disease, Cercospora leaf spot (CLS) disease, and yellow mosaic disease. CLS in mungbean is caused by the fungi *Cercospora canescens* Ellis and Martin and *Pseudocercospora cruenta* (Sacc.) (Deighton 1976). However, *C. canescens* is the most common and severe fungus causing CLS disease in mungbean. This fungus also causes CLS disease in other legumes closely related to mungbean, such as black gram [*Vigna mungo* (L.) Hepper] and cowpea [*Vigna unguiculata* (L.) Walp.] (Duangsong et al. 2016). CLS disease is particularly widespread in the warm wet season. Generally, this disease starts appearing about 30–40 days after planting, depending on the temperature and humidity (Grewal et al. 1980). Disease symptoms of C. *canescens* in mungbean are circular or semi-round brown lesions with pale-tan to gray centers that are surrounded by dark-brown or reddish slightly depressed margins with white masses of spores growing on the dead tissue (Hartman et al. 1993). The disease symptoms initially occur on the upper side of old leaves and progressively spread to the rest of the plant. The spots increase in number and size during flowering, but the increment is most rapid at the pod-filling stage. Without protecting susceptible varieties from the disease, more than 50% losses in seed yield have been reported (Grewal et al. 1980; AVRDC 1984; Iqbal et al. 1995).

Mungbean researchers have long been interested in developing CLS-resistant cultivars. Nonetheless, although several mungbean germplasms possessing resistance to CLS have been reported, such as P-476, P-530, PLM 945, PLM 501, Co 1, 15229, EC 27087-2, EC 27261-3, LGG 463, RMG 429, UPM 92-1 LM-157, T-2, ML-1, ML-3, ML-4, ML-5, ML-9, ML-28, ML-29, ML-23 LM-162, LM-448, 6008-1, PIMS 4, Pusa 105, PDM 15, PDM 2, PDM 113, PDM 115, V1445, V1471, V2273, V4679, V4706, V4717, V4718, V5000, and V5036 (Rath and Grewal 1973; AVRDC 1976; Thakur et al. 1977a, b; Mishra et al. 1988; Tickoo and Satyanarayana 1998; Hartman et al. 1993), only a few germplasms showing highly and stable resistance to CLS have been identified, including V4718 (Hartman et al. 1993). Genetic studies revealed that the CLS resistance in mungbean accessions ML-1, ML-3, EC 27087-2, EC 27261-3, and V4178 is controlled by a single dominant gene (AVRDC 1974; Thakur et al. 1977a, b; Lee 1980; Chankaew et al. 2011). Thakur et al. (1977a) also showed that the gene for CLS resistance is not linked to genes for powdery mildew disease or yellow mosaic disease. However, Mishra et al. (1988) demonstrated that the resistance in accessions Pusa 105, PDM 15, PDM 2, PDM 113, and PDM 115 is controlled by a single recessive gene. Leabwon and Oupadissakoon (1984) reported that the resistance in accessions ML-3, ML-5, ML-15, PML 448, and CES 1D-21 is due to additive gene action. They also noted that broad-sense heritability and narrow-sense heritability of the resistance are high, being 99% and 75%, respectively. However, up until now, only one study has reported gene mapping for CLS resistance in mungbean. Chankaew et al. (2011) performed quantitative trait locus (QTL) mapping for CLS resistance using F_2 and BC_1F_1 populations derived from crosses between the accession V4718 and susceptible cultivar Kamphaeng Saen 1 (KPS1) and found that the resistance is controlled by a single major QTL, *qCLS*. The *qCLS* was mapped on linkage group (LG) 3 between simple sequence repeat (SSR) markers CEDG117 and VR393. It accounted for 65.5 to 80.5% of the disease score variation, depending on season and population. In cowpea [*Vigna unguiculata* (L.) Walp.], a species closely related to mungbean, the resistance to CLS is controlled by a single dominant gene (Fery et al. 1976; Castro et al. 2003), a single recessive gene (Fery et al. 1976; Duangsong et al. 2016, 2018), or oligogenes or polygenes (Booker and Umaharan 2008), depending on resistance sources. A major QTL, *qCLS9.1*, controlling the resistance in cowpea accession IT90K-59-120 was mapped to LG9 (Duangsong et al. 2016). Recently, Heng et al. (2020) finely mapped the *qCLS9.1* and identified two tightly linked genes, *Vigun10g019300* coding for NAD-dependent malic enzyme 1 and *Vigun10g019400* coding for dynamin-related protein 1C, as candidate genes for the resistance. Nonetheless, because the *qCLS9.1* in cowpea and *qCLS* in mungbean are on different LGs, these QTLs for the CLS resistance appear to be non-homologous loci.

Because of the lack of genomic resources for mungbean, the molecular basis of the *qCLS* for CLS resistance in mungbean is still unknown. However, recently, a draft genome sequence of mungbean has become available (Kang et al. 2014). The mungbean genome sequence has enabled DNA marker development and identification of candidate genes for biotic stress resistance in mungbean (Chotechung et al. 2016; Kaewwongwal et al. 2017, 2019). In this paper, we report investigation of molecular basis of the *qCLS* for CLS resistance by fine mapping. The objectives of this study were to (i) finely map the *qCLS* for CLS resistance in the mungbean accession V4718 and (ii) identify candidate gene(s) at the *qCLS*.

Materials and methods

Plant material and DNA extraction

Populations of BC_8F_2 and F_2 developed from crosses between V4718 (donor parent) and KPS1 (recipient parent) were used in this study. V4718 is a landrace mungbean from India. It has been identified as highly resistant to CLS disease (Hartman et al. 1993; Chankaew et al. 2011) and

used in mapping QTL for the resistance (Chankaew et al. 2011). KPS1 is a commercial mungbean variety from Thailand and is susceptible to CLS disease (Chankaew et al. 2011). The BC_8F_2 [KPS1 × (KPS1 × V4718)] population comprised 552 individuals. Procedures for development of this population are shown in Supplementary Fig. S1. The F_2 (KPS1 × V4718) population comprised 155 individuals. It was the same F_2 population previously used by Chankaew et al. (2011) for mapping the *qCLS* locus. The BC_8F_2 and the parental plants were grown under field conditions. Total genomic DNA of parents and BC_8F_2 individuals was extracted from young leaves using the cetyl trimethylammonium bromide method described by Lodhi et al. (1994). The DNA concentration was quantified using a NanoDrop™ 8000 spectrophotometer (Thermo Scientific).

Evaluation of Cercospora leaf spot resistance

The parents and $BC_8F_{2:3}$ families were planted with an augmented design (Federer 1956) from August to October (rainy season) in 2018 at Kasetsart University, Kamphaeng Saen Campus (KU-KPS), Nakhon Pathom, Thailand. Each entry was sown in a single row 1.5 m long with 12.5-cm intra-row and 50-cm inter-row spacing, and two plants per hill (24 plants/row). Inoculation and resistance evaluation of *C. canescens* were conducted following the procedures of Chankaew et al. (2011). In brief, the plants were inoculated by being sprayed with spore suspensions of *C. canescens* (10^4 spores per ml) at 20, 25, and 30 days after planting. Sixty days after planting, plants were scored for CLS reaction using a scale of 1–5, where 1 = no visual disease infection, 2 = 1–25% of leaf area infected, 3 = 26–50% of leaf area infected, 4 = 51–75% of leaf area infected, and 5 = 76–100% of leaf area infected. The average scores for each row were used for statistical and QTL analyses.

CLS disease resistance score data from Chankaew et al. (2011) was used for QTL analysis of the F_2 population in this study. In brief, $F_{2:3}$ progenies and their parents were planted in a randomized complete block design with two replications from August to October of 2008 and 2009 at KU-KPS. Each entry was sown in a single row 2 m long with 12.5-cm intra-row and 50-cm inter-row spacing and two plants per hill (ca. 32 plants/row). Inoculation and disease scoring were the same as described above.

Segregation analysis

To confirm the single-gene inheritance of the CLS resistance in V4718 (AVRDC 1980; Chankaew et al. 2011), $BC_8F_{2:3}$ families with a disease score of 1 or 2 were classified as resistant, and those with a disease score of 3, 4, or 5 were regarded as susceptible. A chi-square (χ^2) test for segregation of CLS disease scores was used to determine the goodness of fit for a 3:1 (resistant/susceptible) ratio. The test was conducted using the R software 2.0.10 (R Development Core Team 2010).

Identification of physical location of the *qCLS* and development of new SSR markers

Previously, the *qCLS* conferring CLS resistance in V4718 was located on LG3 between the SSR markers CEDG117 and VR393 (Chankaew et al. 2011). Thus, we determined the physical location of *qCLS* on the mungbean (VC1973A) reference genome sequence (Kang et al. 2014) by conducting a nucleotide BLAST search of primer sequences of the makers CEDG117 and VR393 against the reference sequence. The genome sequence of 2.88 Mb covering the position of markers CEDG117 and VR393 was downloaded from GenBank (www.ncbi.nlm.nih.gov) and subsequently searched for SSRs using SSRIT software (Temnykh et al. 2001). Primers were designed to amplify the identified SSRs using Primer3 software (Untergasser et al. 2012). The newly developed SSR markers were used to screen for DNA polymorphism between KPS1 and V4718. Polymerase chain reaction (PCR) was carried out on a total volume of 10 µl containing 2 ng of DNA template, 1 × Taq buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 1 U *Taq* DNA polymerase, and 5 pM each of forward and reverse primers. Amplification was conducted on a GeneAmp PCR 9700 system (Applied Biosystems) programed as follows: 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 50–60 °C for 30 s, 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR products were separated by 5% denaturing polyacrylamide gel electrophoresis and visualized by silver staining. Markers showing polymorphism were used to analyze the BC_8F_2 population.

Candidate gene sequencing and development of gene-specific markers

Based on the fine mapping results (see the "Results" section), *LOC106765332* was identified as a candidate gene at the *qCLS* locus for CLS resistance. A mungbean genomic region of ~ 30 Kb covering *qCLS*-flanking markers and LOC106765332 was downloaded from GenBank. Primers were designed to amplify LOC106765332 in KPS1 and V4718 using Primer3 (Untergasser et al. 2012) (Supplementary Table S1). PCR was carried out as described above with the exception that 50 ng of DNA template was used. PCR products were checked with 1.5% agarose gel electrophoresis and then sequenced by Sanger sequencing at TsingKe Biological Technology Co. (Nanjing, China). DNA sequences were assembled manually. The sequences of KPS1 and V4718 were deposited in GenBank (accession numbers MN484602 and MN484603, respectively). The sequences were aligned with the mungbean reference sequence using

Clustal Omega (Sievers et al. 2011) to identify polymorphisms. In addition to the DNA alignment, predicted protein sequences in KPS1 and V4718 were also aligned. Once the DNA sequence polymorphisms were identified, one primer pairs were designed (forward primer: CTCATGAAACCT GGAGAACT and reverse primer: CCCAGTGTACTCAGT TTGACTT) to detect insertion/deletion (InDel) polymorphism between KPS1 and V4718. PCR reaction and amplification were the same as described for SSR marker analysis. The PCR products were separated by 1.5% agarose gel electrophoresis, stained with ethidium bromide, and visualized under ultraviolet light. The InDel marker was used to analyze the BC_8F_2 and F_2 populations.

Linkage and QTL analyses

Genetic linkage maps (LG3) of the F_2 and BC_8F_2 populations were constructed using the software QTL IciMapping 4.1 (Meng et al. 2015). The markers were grouped with a log of odds (LOD) value of 3.0 and ordered based on their positions on the reference genome sequence. Map distance between markers was calculated using Kosambi's mapping function.

The *qCLS* was located on the linkage map by the inclusive composite interval mapping (ICIM) method (Li et al. 2007) implemented in the software QTL IciMapping 4.1. The LOD threshold for the QTL was determined by running a 10,000 permutations test at $P = 0.01$.

Expression analysis of candidate gene

KPS1 and V4718 were grown in 3-inch plastic pots in a greenhouse under natural conditions at KU-KPS in October 2018. Seeds of each accession were grown individually. At growth stage V_3 (Poopakdi et al. 1992), 18 seedlings of each accession were inoculated with *C. canescens* by spraying a spore suspension of approximately 6.0×10^4 spores per ml, while three seedlings were mock-inoculated with water. Leaf samples (first and second trifoliolate leaves) were collected 0, 2, 4, 6, 12, 24, and 48 h after inoculation. The leaves were immediately frozen in liquid nitrogen and stored in a freezer at − 80 °C. Total RNA was isolated from the leaves using the method described by Laksana and Chanprame (2015). The concentration and qualification of RNA was quantified using a NanoDrop™ 8000 spectrophotometer (Thermo Scientific). cDNA was synthesized from the RNA template using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions. Primers specific to coding sequence (CDS) of *LOC106765332* and two internal reference genes, including *VrACTIN-1* (actin-related protein 1; GenBank accession no. *LOC106770112*) and *VrCYP20* (peptidyl-prolyl cis–trans isomerase) (Li et al. 2015), were designed for quantitative reverse transcription PCR (qRT-PCR) analysis using Primer3 (Untergasser et al. 2012) (Supplementary Table S2). The qRT-PCR analysis was conducted using a SensiFAST™ SYBR® No-ROX kit (BIOLINE). Three biological replicates, each with three technical replicates, were performed for KPS1 and V4718 on a CFX96™ Real-Time system (BIO-RAD) using the following thermal cycle: 95 °C for 3 min, followed by 40 cycles of 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 30 s. Quantification of gene expression was carried out using the relative $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). Significant differences in level of gene expression between KPS1 and V4718 every hour after inoculation were tested by *t* test at 1% probability using the R program, version 2.10.0.

Results

CLS resistance in the parent and BC_8F_2 populations

KPS1 was susceptible to CLS disease with an average disease score of 3.2, whereas V4718 was immune to CLS disease with an average disease score of 1. The BC_8F_2 population with 552 individuals showed a disease score from 1 to 5 with an average of 2.0. Frequency distribution of the CLS disease score in the BC_8F_2 population showed continuous segregation, with skewing toward V4718 (Fig. 1).

Four hundred and thirty-six lines of $BC_8F_{2:3}$ families were classified as resistant, whereas 116 $BC_8F_{2:3}$ families were susceptible. The χ^2 test revealed that the segregation of CLS resistance in the BC_8F_2 population fitted well with a 3:1 (resistant/susceptible) ratio at $P = 0.01$ ($\chi^2 = 4.67$, $P = 0.31$). This suggested that the CLS disease resistance in V4718 is controlled by a single dominant gene.

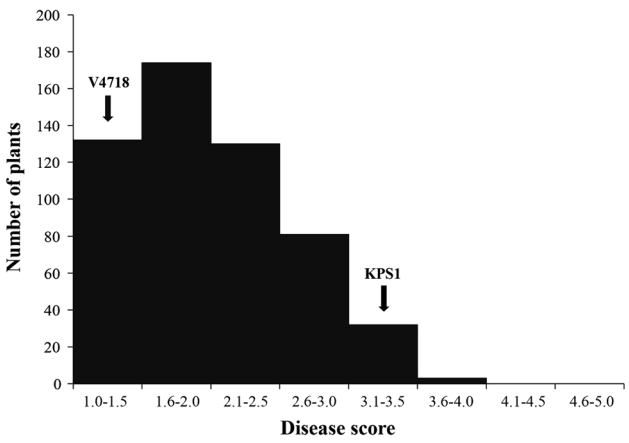

Fig. 1 Frequency distribution of Cercospora leaf spot disease score in BC_8F_2 population [(KPS1 × V4718) × KPS1] evaluated during wet season of 2018

Location of the *qCLS* on mungbean reference genome and new SSR markers

A nucleotide BLAST search of the SSR primer sequences of CEDG117 and VR393 flanking the *qCLS* (Chankaew et al. 2011) gene against the mungbean reference sequence revealed that these markers are at the positions 31,550,068 and 34,426,801 on chromosome 6. Thus, CEDG117 and VR393 were 2.88 Mb apart. Based on the GenBank gene annotation, 199 annotated genes exist in this region.

We developed 133 new SSR markers located between and around the CEDG117-VR393 marker region (Supplementary Table S3). All of the markers successfully amplified the DNA of KPS1 and V4718, but only 16 of them were polymorphic. The polymorphic markers, together with CEDG117 and VR393, were used for genotyping the BC_8F_2 population developed in this study, while the 16 new polymorphic markers were also used for genotyping the F_2 population. In addition, InDel marker VrTAF5-indel (see details in the section "Sequencing of *VrTAF5* and development of gene-specific marker") was also included in genotyping of the BC_8F_2 and F_2 populations.

Fine mapping of *qCLS*

Nineteen polymorphic markers, including the 16 newly developed SSR markers and 1 new InDel marker, together with CEDG117 and VR393, were used to construct a genetic linkage map for the F_2 and BC_8F_2 populations. All 19 markers were clustered in the same LG3, as observed in the previous study (Chankaew et al. 2011). The LG of the F_2 and BC_8F_2 populations spanned a length of 12.1 and 18.7 cM, respectively (Fig. 2).

QTL analysis by ICIM in the F_2 population using CLS disease scores in both years 2008 and 2009 consistently revealed that the *qCLS* for the resistance was located at 5.6 cM between markers Vr6gCLS085 and VrTAF5_indel (Fig. 2a). For the disease score in 2008, the *qCLS* accounted for 66% of the disease resistance variation and showed additive and dominant effects of 1.0 and − 0.4, respectively. In case of the disease score in 2009, the *qCLS* explained 52% of the disease resistance variation with an additive effect of 0.9 and dominant effect of 0.2.

In the BC_8F_2 population for which the resistance was evaluated in 2018, ICIM showed that the *qCLS* was located at 5.9 cM between markers Vr6gCLS085 and VrTAF5_indel (Fig. 2b). The *qCLS* accounted for 56% of the disease resistance variation and showed additive and dominant effects of 0.6 and < 0.01, respectively.

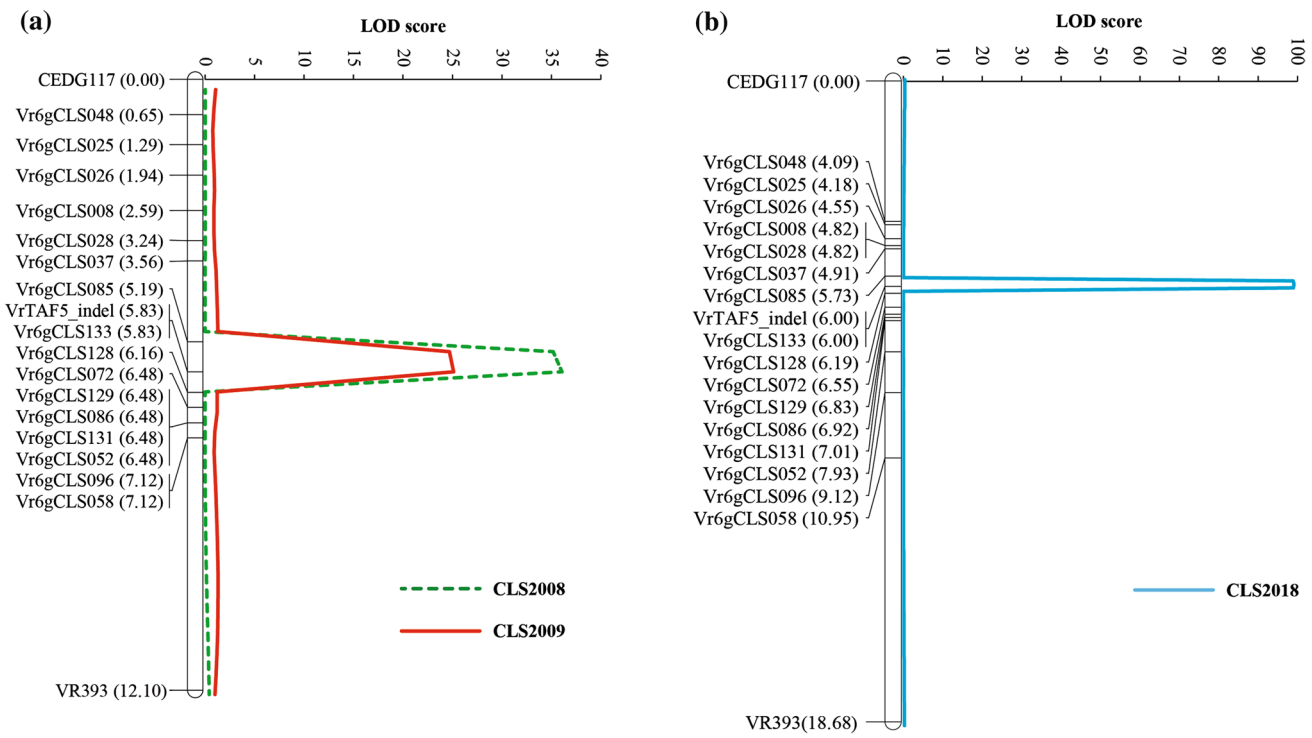

Fig. 2 Logarithm of odds (LOD) graphs for the major QTL controlling Cercospora leaf spot disease resistance detected on chromosome 6 by composite interval mapping in F_2 (KPS1 × V4718) (**a**) and BC_8F_2 [(KPS1 × V4718) × KPS1] (**b**) populations

A comparative linkage map comparing the locations of the *qCLS* detected in this study and in the study of Chankaew et al. (2011) is shown in Fig. 3. The map also illustrates the location of the *qCLS* on the mungbean reference genome. The physical distance of the markers Vr6gCLS085 and VrTAF5_indel/Vr6gCLS133 that flank the *qCLS* on the mungbean reference genome was only ~ 13 Kb. The markers were at the positions 32.607 and 32.620 Mb on mungbean chromosome 6 (Fig. 3), respectively. Based on the position of the *qCLS* on the reference genome, *qCLS* corresponded to *LOC106765332* encoding general transcription initiation factor IID (TFIID) subunit 5, or TATA-box-binding protein (TBP)-associated factor 5 (TAF5). We designated LOC106765332 as *VrTAF5*.

Sequencing of *VrTAF5* and development of gene-specific marker

Based on the mungbean reference sequence (VC1973A), *VrTAF5* has a genomic sequence length of 10.228 Kb with a CDS length of 1.980 Kb from 19 exons. The *VrTAF5* protein sequence length is 659 amino acid residues. We sequenced *VrTAF5* in KPS1 and V4718 and aligned the sequences with VC1973A. Alignment of the open reading frame revealed size differences and nucleotide polymorphisms among the three mungbean accessions (Supplementary Fig. S2). The sequence length of KPS1 was the same as that of VC1973A (10,228 bp), while that of V4718 was shorter (10,195 bp) than that of KPS1 and VC1973A. In total, as many as 62 polymorphisms (51 SNPs and 11 InDels) were identified among the sequences (Supplementary Fig. S2). Forty-three of the SNPs and all of the InDels were in introns. Each of eight SNPs were in different exons (Fig. 4), including SNPs at the position 1517 in exon 2, 1739 in exon 3, 4932 in exon 8, 5916 in exon 12, 9548 in exon 16, 9884 in exon 17, 10,010 in exon 18, and 10,228 in exon 19 (Fig. 4). Among these SNPs, the SNP in exon 12 was unique to VC1973A, while the other seven SNPs were diagnostic between KPS1 and V4718. These positions corresponded to the positions 165, 240, 749, 1127, 1587, 1812, 1857, and 1980 of the CDS of the *VrTAF5*. Alignment of CDSs of the *VrTAF5* in KPS2, V4718, and VC1973A is shown in Supplementary Fig. S3. Among the eight SNPs, only those at positions 749 and 1127 caused amino acid changes in the VrTAF5 protein.

When the predicted protein sequences of VrTAF5 in KPS2, V4718, and VC1973A were aligned (Fig. 5), polymorphisms were found at residue positions 250 and 376. At residue position 250, KPS2 and VC1973A had the same amino acid [serine (S)], but different from V4718 [threonine (T)]. At residue 376, VC1973A had an amino acid [histidine (H)] different from that of KPS1 and V4718, both of which had the same amino acid [arginine (R)].

An InDel marker, VrTAF5-indel, was developed to detect a 24-bp InDel polymorphism in the *VrTAF5* gene between KPS1 and V4718. The marker VrTAF5-indel showed polymorphism between the two mungbean cultivars using 2% agarose gel electrophoresis (Fig. 6a). The marker was used to analyze the F_2 and BC_8F_2 populations for fine mapping of the *qCLS* locus (Fig. 6b). The marker was co-segregated

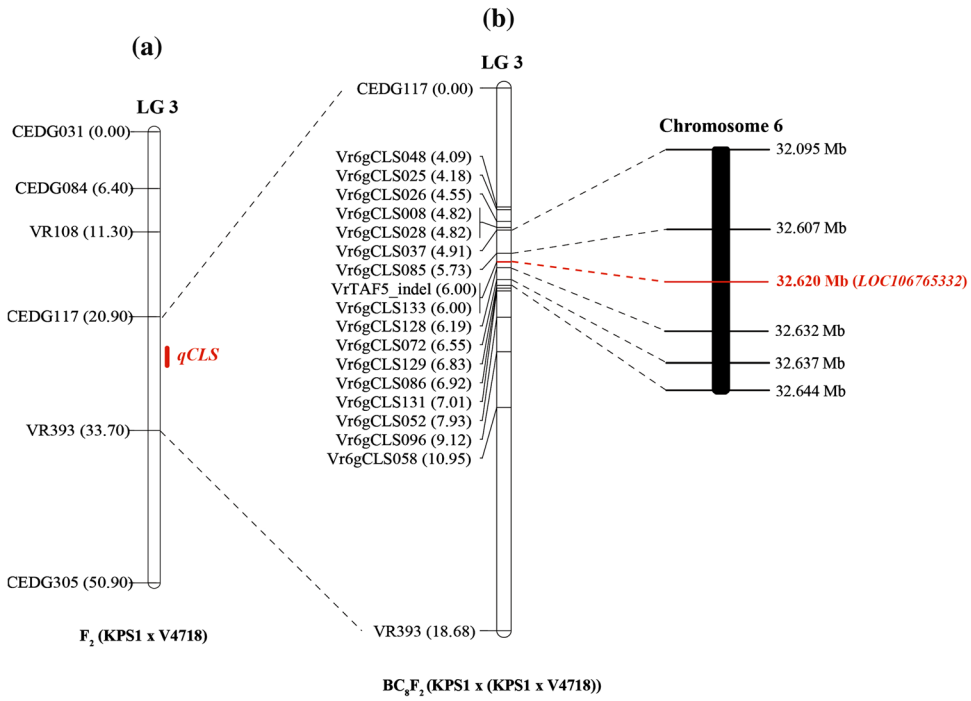

Fig. 3 A comparative genome map illustrating *qCLS* reported by Chankaew et al. (2010) (**a**) and detected by this study (**b**) and the location of *qCLS* on chromosome 6 of the mungbean reference genome (VC1973A). Numbers in parentheses are marker positions on the linkage map in centimorgans (cM)

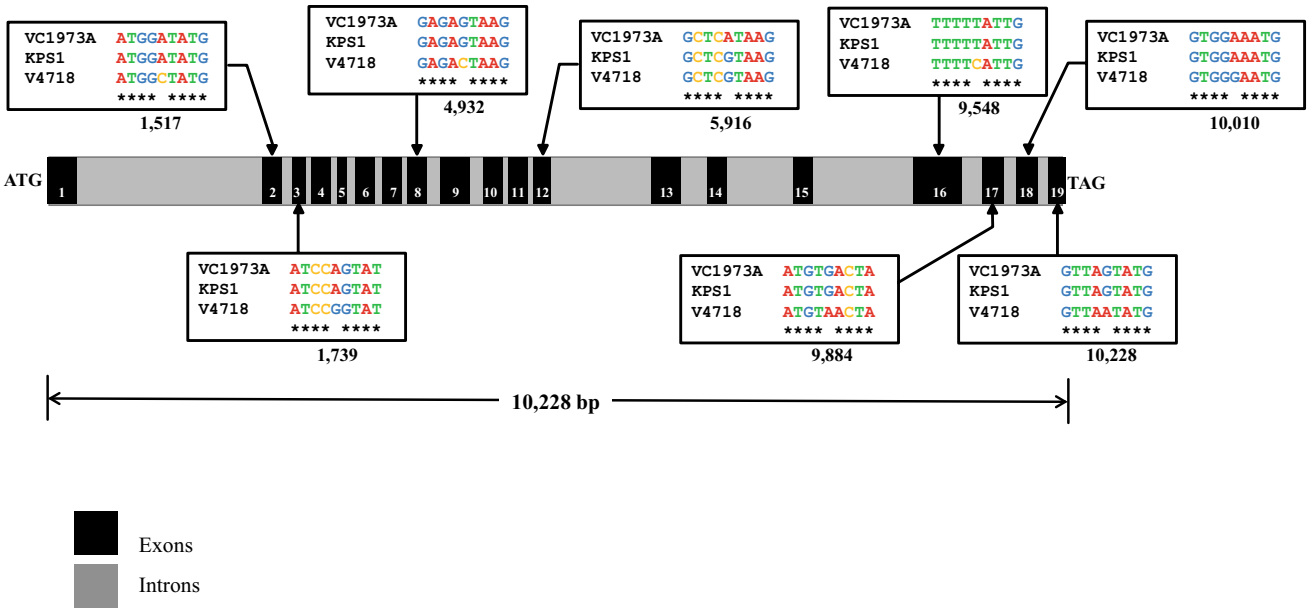

Fig. 4 Schematic showing the location of mutations in exons of *VrTAF5* (*LOC106765332*) in mungbean accessions KPS1 and V4718 and reference sequence VC1973A. The start codon (ATG), 19 exons (solid boxes), 18 introns (solid line), and the stop codon (TAG) are indicated

with SSR marker Vr6gCLS133, which is located in *VrTAF5* and tightly linked to the *qCLS*.

Expression of *VrTAF5* in KPS1 and V4718

Relative expression levels of *VrTAF5* in KPS1 and V4718 in unifoliate leaves inoculated or not inoculated with *C. canescens* was determined by qRT-PCR 0, 2, 4, 6, 12, 24, and 48 h after inoculation with two internal reference genes, *VrACTIN-1* (Fig. 7a) and *VrCYP20* (Fig. 7b). qRT-PCR revealed that expression levels of *VrTAF5* in pathogen-inoculated leaves of KPS1 and V4718 at 0, 2, 4, 6, 12, 24, and 48 h after inoculation were low and not statistically different ($P > 0.01$). In addition, the expression of *VrTAF5* in the pathogen-inoculated leaves was not statistically different from that in the mock-inoculated leaves ($P > 0.01$).

Discussion

Although CLS is a common disease of mungbean grown in tropical and sub-tropical regions and germplasm possessing CLS resistance exists, only one study has reported molecular genetics and molecular breeding of this disease in mungbean. Mungbean accession V4718 has been found to be highly and stably resistant to CLS (Hartman et al. 1993; Chankaew et al. 2011). A major QTL, *qCLS*, conferring CLS resistance in V4718, was mapped to the mungbean genome nearly 9 years ago, despite the distance between flanking markers of the *qCLS* being very large (> 10 cM) (Chankaew et al. 2011). However, due to a lack of genomic resources, such as efficient DNA markers and a high-density linkage map, no additional effort has been made to further investigate the *qCLS*. By exploiting the recently released mungbean genome sequence (Kang et al. 2014) for DNA marker development, QTL mapping, and bioinformatics analysis, we were able to finely map the *qCLS* and identify a candidate gene at the *qCLS* in V4718.

It has been shown that the CLS resistance in the mungbean V4718 is controlled by a single dominant gene (Lee 1980; Chankaew et al. 2011). *qCLS* has been identified as the only locus conferring CLS resistance in V4718 (Chankaew et al. 2011). In the present study, BC_8F_2 was developed by phenotypic selection and marker-assisted selection (MAS) using flanking markers CEDG117 and VR393. The segregation of the BC_8F_2 lines for CLS was 3:1 for resistant and susceptible, confirming that the resistance in V4718 is controlled by a single dominant gene.

Previously, *qCLS* was localized between markers CEDG117 and VR393 (Chankaew et al. 2011). In the mungbean reference genome (VC1973A; Kang et al. 2014), the physical distance between these two markers is 2.88 Mb on chromosome 6 and this region contains numerous annotated genes. In our study, QTL mapping in both BC_8F_2 and F_2 populations narrowed down the *qCLS* to a genome region of only about ~ 13 Kb delimited by markers Vr6gCLS085 and VrTAF5_indel/Vr6gCLS133 (Fig. 3). There was only one annotated gene in this genome region, *VrTAF5*, which encodes TAF5 (Fig. 5). DNA and protein sequence alignments revealed that an SNP causes an amino acid change

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VC1973A     MDEDQIEGCVSGYLKQKGFAQKDDQLQLSNADSSLQPDTLNRAQLERGSARYHDGYGRLR   60
KPS1        MDEDQIEGCVSGYLKQKGFAQKDDQLQLSNADSSLQPDTLNRAQLERGSARYHDGYGRLR   60
V4718       MDEDQIEGCVSGYLKQKGFAQKDDQLQLSNADSSLQPDTLNRAQLERGSARYHDGYGRLR   60
            ************************************************************

VC1973A     SWAYRSLESYKHELLRVLYPVFVHCFMDLVAKGHLQEAWNFFNTFREDHEMLHSRDLQKL   120
KPS1        SWAYRSLESYKHELLRVLYPVFVHCFMDLVAKGHLQEAWNFFNTFREDHEMLHSRDLQKL   120
V4718       SWAYRSLESYKHELLRVLYPVFVHCFMDLVAKGHLQEAWNFFNTFREDHEMLHSRDLQKL   120
            ************************************************************

VC1973A     GLVLSPTHLEEMEFAHSLRQSKFNIKICRYSYELLLQHLHSMQSTTIIGIINEHINFQVT   180
KPS1        GLVLSPTHLEEMEFAHSLRQSKFNIKICRYSYELLLQHLHSMQSTTIIGIINEHINFQVT   180
V4718       GLVLSPTHLEEMEFAHSLRQSKFNIKICRYSYELLLQHLHSMQSTTIIGIINEHINFQVT   180
            ************************************************************

VC1973A     AGQPSSISDDPEAVTLSGSIQDAVNQINQKEILWGMFEDSVEDHVDKAGALLSGTEKGEG   240
KPS1        AGQPSSISDDPEAVTLSGSIQDAVNQINQKEILWGMFEDSVEDHVDKAGALLSGTEKGEG   240
V4718       AGQPSSISDDPEAVTLSGSIQDAVNQINQKEILWGMFEDSVEDHVDKAGALLSGTEKGEG   240
            ************************************************************

VC1973A     EGKEGDNDESKKRSIDVGKQGNSVKKVKKDKVSSATGKNAKPEATTISAAPRIKPEIPLP   300
KPS1        EGKEGDNDESKKRSIDVGKQGNSVKKVKKDKVSSATGKNAKPEATTISAAPRIKPEIPLP   300
V4718       EGKEGDNDETKKRSIDVGKQGNSVKKVKKDKVSSATGKNAKPEATTISAAPRIKPEIPLP   300
            *********:**************************************************

VC1973A     IVSTDVELSILDDLRNRVQLSSVALPSVNFYTFVNTHNGLSCSSISHDGSLIVGGFSDSS   360
KPS1        IVSTDVELSILDDLRNRVQLSSVALPSVNFYTFVNTHNGLSCSSISHDGSLIVGGFSDSS   360
V4718       IVSTDVELSILDDLRNRVQLSSVALPSVNFYTFVNTHNGLSCSSISHDGSLIVGGFSDSS   360
            ************************************************************

VC1973A     LKVWDMAKLEQQSTAHFSQGGNDMSQNEQIIGQNSGRRQYTLYQGHSGPVYAATFSAAGD   420
KPS1        LKVWDMAKLEQQSTARFSQGGNDMSQNEQIIGQNSGRRQYTLYQGHSGPVYAATFSAAGD   420
V4718       LKVWDMAKLEQQSTARFSQGGNDMSQNEQIIGQNSGRRQYTLYQGHSGPVYAATFSAAGD   420
            ***************:********************************************

VC1973A     FLLSSSADKTVRLWSTKLNANLVCYKGHNYPIWDVQFSPAGHYFASCSHDRTARIWSMDR   480
KPS1        FLLSSSADKTVRLWSTKLNANLVCYKGHNYPIWDVQFSPAGHYFASCSHDRTARIWSMDR   480
V4718       FLLSSSADKTVRLWSTKLNANLVCYKGHNYPIWDVQFSPAGHYFASCSHDRTARIWSMDR   480
            ************************************************************

VC1973A     TKPLRIMAGHLSDVDCVQWHPNCNYIATGSSDKTVRLWDVQSGECVRVFIGHRSMILSLA   540
KPS1        TKPLRIMAGHLSDVDCVQWHPNCNYIATGSSDKTVRLWDVQSGECVRVFIGHRSMILSLA   540
V4718       TKPLRIMAGHLSDVDCVQWHPNCNYIATGSSDKTVRLWDVQSGECVRVFIGHRSMILSLA   540
            ************************************************************

VC1973A     MSPDGRYMASGDEDGTIMMWDLSSGCCVTPLVSHTSCVWSLAFSCEGSLLASGSADCTVK   600
KPS1        MSPDGRYMASGDEDGTIMMWDLSSGCCVTPLVSHTSCVWSLAFSCEGSLLASGSADCTVK   600
V4718       MSPDGRYMASGDEDGTIMMWDLSSGCCVTPLVSHTSCVWSLAFSCEGSLLASGSADCTVK   600
            ************************************************************

VC1973A     FWDVTTGIKVPRNEENRSGNANRLRSLKSLPTKSASVYSLQFSRRNLLFAAGAVAKSGC*   659
KPS1        FWDVTTGIKVPRNEENRSGNANRLRSLKSLPTKSASVYSLQFSRRNLLFAAGAVAKSGC*   659
V4718       FWDVTTGIKVPRNEENRSGNANRLRSLKSLPTKSASVYSLQFSRRNLLFAAGAVAKSGC*   659
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Fig. 5 Alignment of amino acid sequences in VrTAF5 protein. Sequences from Cercospora leaf spot resistant V4718 and susceptible KPS1 are shown along with the reference sequence (VC1973A). An asterisk indicates an amino acid change unique to V4718

in V4718, as compared with KPS1 (Figs. 4 and 5). These results together indicated that *VrTAF5* is responsible for CLS resistance in mungbean accession V4718. Because gene expression analysis showed no significant expression of *VrTAF5* in V4718 and KPS1 (Fig. 7), this indicated that the expression of *VrTAF5* is not triggered by *C. canescens* infection and that the CLS resistance in V4718 is likely due to a change in the VrTAF5 protein structure and thus its function. In rice, two SNPs in *xa5* causes an amino acid change but not expression of the gamma subunit of general transcription factor TFIIA (TFIIAγ), which produces the resistance to *Xanthomonas oryzae* pv. *oryzae* (Lyer and McCouch 2004). TAFs are a key component in the general transcription factor TFIID complex (Tora 2002). Furthermore, TFIID plays a central role in the recognition of core promoter elements and is an essential for accurate transcription initiation by RNA polymerase II in eukaryotic genes. It is a multi-protein complex composed of TBP and 12–15 TAFs that are highly

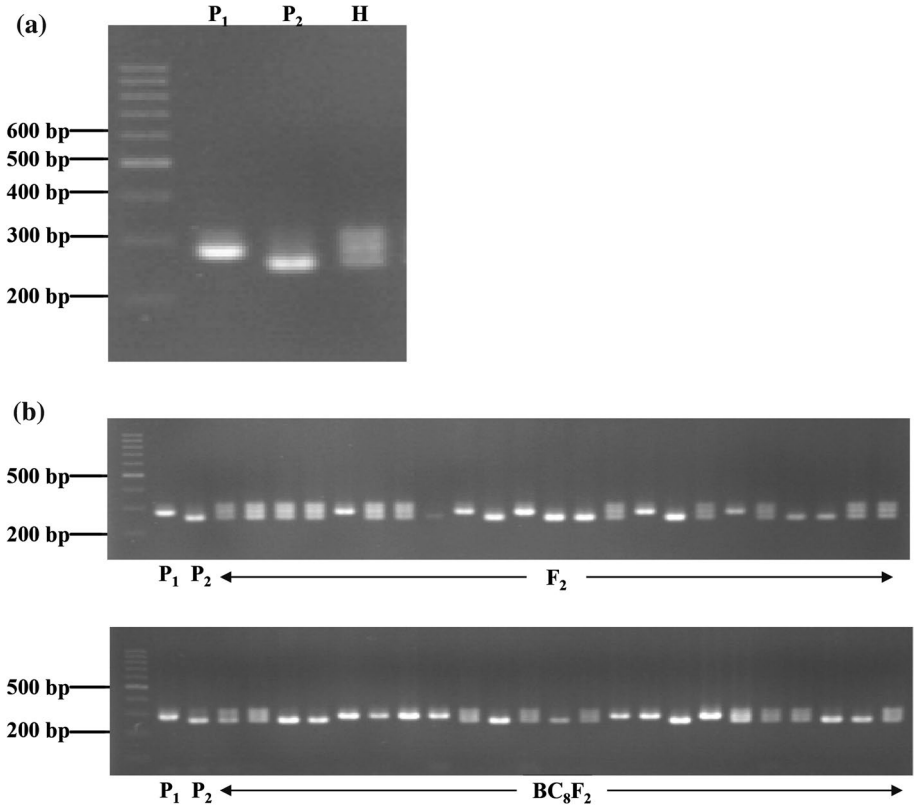

Fig. 6 DNA bands corresponding to regions amplified by VrTAF5_indel markers specific to 24 bp deletion in intron 7 of *VrTAF5* (*LOC106765332*) in V4718. DNA amplification bands obtained with InDel markers between parent (**a**) and F_2 and BC_8F_2 (**b**) populations

conserved (Tora 2002). It has been shown that TAFs play multiple functions within the TFIID holo-complex (Chalky and Verrijzer 1999; Verrijzer et al. 1994, 1995; Burke and Kadonaga 1997; Imhof et al. 1997; Dikstein et al. 1996; Mizzen et al. 1996; Kouzarides 2000) and are involved in transcription and interactions with gene-specific activators and other general transcription factors, either stabilizing the preinitiation complex (Roeder 1996; Patel et al. 2018) or inducing changes in them (Oelgeschkager et al. 1996). TAFs are also a key component of histone acetyltransferase (HAT) complexes, such as the Spt-Ada-Gcn5 acetyltransferase (SAGA) complex. SAGA is a transcriptional co-activator complex functioning in nucleosome histone acetylation and chromatin-associated transcriptional activation or suppression (Grant et al. 1998; Pray-Grant et al. 2002). The SAGA complex has been shown to be involved in stress resistance in plants (reviewed in Moraga and Aquea 2015). A recent study with *Arabidopsis* demonstrated that TAF15b contributed to a nucleotide-binding-domain leucine-rich repeat immunity system through posttranscriptional mechanisms (Dong et al. 2016). TAF5 is the basis for the formation of both the TFIID and SAGA complexes where its dimer forms a scaffold for the assembly of different TAFs in the complexes (Leurent et al. 2004; Scheer et al. 2012) or modulating interaction between other TAFs (Scheer et al. 2012). TAF5 is an organization center for core subunit assemblies for the TFIID complex (Kolesnikova et al. 2018), reducing TAF5 by gene knockout and resulting in the degradation of all other TAFs except TAF2 (Wright et al. 2006). A study of *Arabidopsis* revealed that *TAF5* is an important gene involved in male gametogenesis, pollen tube growth, and transcriptional mechanisms for the maintenance of indeterminate inflorescence (Mougiou et al. 2012). Although no direct association between TAF5 and disease resistance in plants has been reported to date, one study identified hyperacetylation by acetylome profiling in maize plants treated with effector HC-toxin (HTC), a histone deacetylase inhibitor (HDACi), produced by the fungal pathogen *Cochilobolus carbonum* race 1, HTC-producing strain of *C. carbonum* race 1 (Tox^+), or HTC-deficient strain of *C. carbonum* race 1 (Tox^-). The study revealed that hyperacetylation events mainly occurred with either HCT or Tox^+ and that the majority of hyperacetylated proteins, including TAF5, TAF6, gene-specific transcription factors, transcription corepressors, chromatin remodeling enzymes, and HAT enzymes, are transcriptional regulatory proteins (Walley et al. 2018). The authors have shown that the hyperacetylation of these proteins may affect the transcriptional response of the host plant during pathogen infection, resulting in the promotion of pathogen virulence. Nonetheless, these results suggested that TAF5 plays a role in mediating the response of plants to disease, although how TAF5 contributed to the resistance is not known. In a case similar to that of *VrTAF5*, *xa5* gene encoding for the gamma subunit of general transcription factor TFIIA has

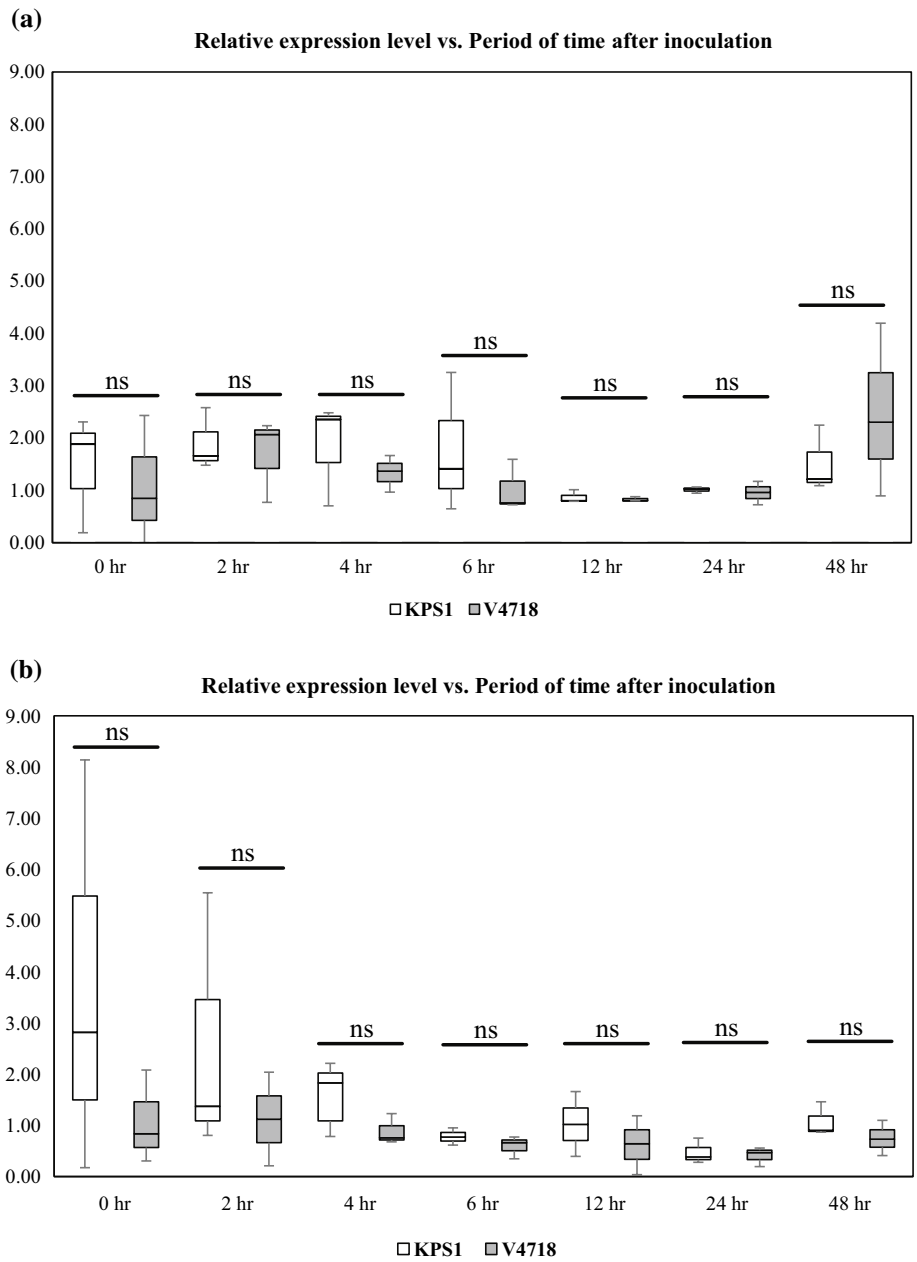

Fig. 7 Relative expression levels of *VrTAF5* (*LOC106765332*) in KPS1 and V4718 inoculated with *C. canescens* using *VrACTIN-1* (**a**) and *VrCYP20* (**b**) as the reference genes. Three biological replicates, with three technical replicates, were performed. Error bars indicate standard error of the mean. The significant differences in expression levels of *Vradi06g13500* between KPS1 and V4718 at seven time points were determined with a *t* test at $P = 0.01$

been found to confer resistance to bacterial blight disease caused by *X. oryzae* pv. *oryzae* in rice (Lyer and McCouch 2004).

Plants and other organisms have one *TAF5* gene (Mougiou et al. 2012). In *Arabidopsis*, the *AtTAF5* protein has two conserved functional domains, which are the NTD2 domain in the N-terminus and the WD40-repeat domain in the C-terminus. The NTD2 domain plays an essential role in recognizing the TFIID complex by dimerization (Bhattacharya et al. 2007), while the WD40-repeat domain forms a beta-propeller structure (Smith et al. 1999) that is critical for the structural role of TAF5 in the TFIID and SAGA complexes (Durso et al. 2001; Leurent et al. 2004) and has been shown to mediate protein–protein interaction (Tao et al. 1997). The composition and integrity of TFIID and SAGA complexes are affected by mutation(s) in the WD40-repeat domain of TAF5 (Durso et al. 2001). However, in our study, sequence alignment between AtTAF5 and VrTAF5 revealed that the S250T amino acid change in mungbean varieties KPS1 and V4718 is neither in the NTD2 domain nor the WD40-repeat domain, but within a linker region between the two domains (Fig. 8). Interestingly, in AtTAF5, a stretch of acetylated amino acid residues was found in the linker region upon HDACi (apicidin and trichostatin A) treatment of leaves (Hartl et al. 2017). HDACi can alter gene transcription by chromatin remodeling and change the structure of proteins

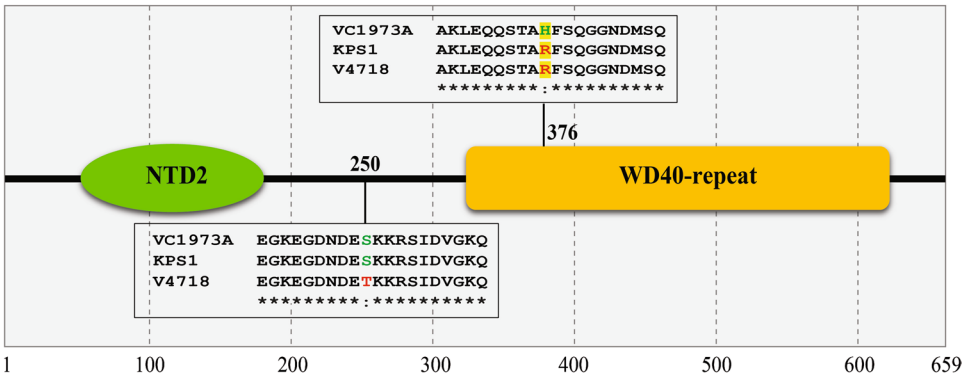

Fig. 8 Predicted domain organization of VrTAF5 protein

in transcription factor complexes (Gui et al. 2004). Based on this information, the amino acid change in the TAF5 linker region of V4718 appears to play a role in the resistance to *C. canescens*, possibly by invoking a transcriptional response in the host plant through histone acetylation. Further study is needed to address how the amino acid change in V4718 contributes to *C. canescens* resistance.

Frogeye leaf spot disease caused by *Cercospora sojina* K. Hara is an important disease of soybean (*Glycine max* (L.) Merr.), a legume crop closely related to mungbean. Gene mapping studies revealed that genes/QTLs controlling *C. sojina* locates on soybean chromosomes 13 (Hoskins 2011; Pham et al. 2015), 16 (Hoskins 2011; Mian et al. 1999) and 18 (Hoskins 2011). Since the Cercospora leaf spot in mungbean and the frogeye leaf spot in soybean are both caused by *Cercospora* fungi, it is possible that genes for the resistance in these two crops are conserved. However, BLASTP search using protein sequence of *VrTAF5* (*LOC106765332*) against soybean genome sequence (https://phytozome-next.jgi.doe.gov/info/Gmax_Wm82_a4_v1) revealed that this gene corresponds to *Glyma.17G222200* (Gm17:37410984.0.37431412; score = 1189.87, *E* value = 0.0 and identity = 90%) locating on chromosome 17. This indicates that the resistance to *C. canescens* in mungbean and the resistance to *C. soina* in soybean is not conserved. It is noteworthy that three candidate genes including *Glyma13g25320*, *Glyma13g25340* and *Glyma13g25350* have been identified for the resistance genes/QTLs on the soybean chromosome 13 (Pham et al. 2015). Among them, *Glyma13g25350* is the most probable candidate gene for the resistance because *Glyma13g25350* is a heterotrimeric G-protein (www.soybase.org) which plays a central role in plant signal transduction involving in programmed cell death in plant immunity to pathogens (Zhang et al. 2012).

Although three to four crops of mungbean can be grown per year, progress in breeding for CLS disease resistance is limited because the disease occurs only during the wet season. This is a limiting factor in traditional breeding based on phenotypic selection. InDel and SSR markers are standard DNA marker systems for MAS because of their high polymorphism, simple detection, and high reproducibility. In this study, the InDel marker VrTAF5_indel and SSR marker Vr6gCLS133 were developed from the intergenic region of the candidate gene *VrTAF5* controlling the CLS resistance and shown to be co-segregated perfectly with the resistance (Fig. 6). These markers would ease and accelerate development of new mungbean cultivar(s) resistant to the CLS disease by year-round selection through MAS.

Acknowledgements We are thankful to the Joint Legume Research Center between Jiangsu Academy of Agriculture Sciences and Kasetsart University for molecular lab facilities.

Author contributions statement PS conceived and designed the study. CY conducted all of the experiments. JC and XY participated in marker, DNA sequencing, and gene expression analyses. CY, PS, and SC developed the populations and conducted phenotyping. XC and PS acquired funding. PS and CX supervised the study. CY, PS, and XC wrote the manuscript. PS edited and revised the manuscript. All authors reviewed the manuscript.

Funding This work was supported by the National Key Research and Development Program of China (Grant No. 2016YFE0203800), the China Agricultural Research System (CARS-08), and the Jiangsu Agriculture Industry Technology System (Grant No. JATS[2018]255).

Availability of data and materials All information is specified in the manuscript or included as Additional Files.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding authors declare that they have no conflict of interest.

Ethical statement The authors declare that this research has no human and animal participants and that the experiments comply with the current laws of the country in which they were carried out.

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