

Transcriptome wide identification of CDR family in *Citrus latifolia* and its expression during HLB infection

Felipe Roberto Flores-de la Rosa

Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias

Carlos González-Cruz

Tecnológico Nacional de México, Campus Ursulo Galván

Jacel Adame-García

Tecnológico Nacional de México, Campus Ursulo Galván

Arianna Chan-León

Centro de Investigación Científica de Yucatán

Ricardo Santillán-Mendoza

Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias

Jorge M. Santamaría

Centro de Investigación Científica de Yucatán

Humberto Estrella-Maldonado (✉ estrella.humberto@inifap.gob.mx)

Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias

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Abstract

Huanglongbing (HLB) is the most important disease in citrus production around the world. Currently, there are not HLB-resistant citrus species or genotypes, however, Persian lime (*Citrus latifolia* Tan.) has been reported as the most tolerant citrus species to this disease, but their molecular mechanisms of tolerance has not being characterized yet. In other citrus species, *Poncirus trifoliata*, it has been suggested that the overexpression of *CDR* genes is associated to HLB tolerance, therefore, the aim of this work was to identify *CDR* gene family members in the Persian lime transcriptome and to determine if the HLB infection induces their expression. A Bayesian approach was used to search transcripts with one or two catalytic domains, then, a non-weighted parsimony phylogeny for *CDR* genes was carried out with the selected transcripts from *C. latifolia*, *P. trifoliata* and *C. sinensis*, to identify phylogenetic homologous. The corresponding encoded proteins were obtained from the selected genes, and the similarity among them was estimated using alignments, identity matrix, UPGMA and conserved motifs analysis. Once the *CICDR* genes were identified, their expression was determined with the transcriptome data and validated by RT-PCR and qRT-PCR in tissues of asymptomatic and HLB-symptomatic leaves. 146 sequences with one or two catalytic domains, were found, and 7 *CDR* clades were recovered. Similarity analysis demonstrated that there is a complex relationship among *CDR* genes and proteins in the species used in the present study. Moreover, the transcriptome and qRT-PCR data showed that none of the *CICDR* genes were expressed, neither in healthy nor in HLB-infected Persian lime plants, except for the *CICDR8a* gene, which showed high expression. However, no significant differences were found between the *CICDR8a* REL values from symptomatic or non-symptomatic leaves. Our results demonstrated the presence of *CDR* genes in the Persian lime genome, however, they suggest that HLB tolerance is not correlated with the overexpression of this gene family in this species.

Introduction

Huanglongbing (HLB), also called citrus greening disease, represents the worse threat in the history of citrus industry. It is a disease caused by three different species of *Candidatus Liberibacter* (Thapa et al. 2020), being *Ca. L. asiaticus* (CLas) the most dispersed worldwide (Ajene et al. 2020) and the most reported in citrus plants and psyllids in Mexico (López-Buenfil et al. 2017). Mexican citrus (mainly lemons and limes) are exported to various countries around the world, (USDA, 2020). The Mexican state Veracruz is the most important producer and exporter of Persian lime (*Citrus latifolia* Tan.) in the world, due to the high quality of the harvested fruits (Berdeja-Arbeu et al. 2019). However, the HLB symptomatology has been observed in Persian lime fields in Veracruz, corroborating the CLas presence (Marquez-Pérez et al. 2018).

Although HLB-infected Persian lime plants show physiological alterations such as starch accumulation and decreased chlorophyll contents (Flores-de la Rosa et al. 2021), and economic losses have been also reported (Flores-Sánchez et al. 2015). Persian lime is still considered one of the least susceptible to HLB when compared to sweet citrus such as orange (*C. sinensis*), tangerine (*C. clementina*), and even to other limes such as Mexican lime (*C. aurantifolia*), as Persian lime trees are able of continue their production for many years after CLas has been detected (Evans et al. 2014). Recently, HLB diseased Persian lime trees maintained high physiological performance in terms of photosynthesis, stomatal conductance and transpiration (Sivager et al. 2021), suggesting that phloem activity can be maintained in this species during HLB infection.

HLB tolerance has been well documented in citrus such as *Poncirus trifoliata* (Ramadugu et al. 2016) and its hybrids with other citrus species (Albretch and Bowman, 2012). *Poncirus trifoliata* is a very important species in the citrus industry because it is used as a rootstock to other different citrus species scions, inducing very desirable features to the fruits (Hu et al. 2022). Transcriptomic and genomic studies have suggested that tolerance to HLB in *P. trifoliata*, is associated to the overexpression of *CDR* (*Constitutive Diseases Resistance*) family genes, these genes were highly overexpressed in tolerant genotypes, but absent in susceptible ones (Rawat et al. 2017). *CDR* gene family has being characterized in *Arabidopsis thaliana*, as belonging to aspartic proteases enzymes (Xia et al. 2004), which have been associated to defense responses in plants against both biotic and abiotic stresses (Figuereido et al. 2021). However, this gene family has not been studied in Persian lime in association to HLB tolerance. Therefore, the aim of the present study was to identify *CDR* family members in the Persian lime transcriptome and to determine the effect of HLB-infection on the expression of *CDR* gene family.

Materials And Methods

Identification of *CDR-like* sequences in the Persian lime transcriptome

We obtained all *CDR*-like transcripts from a transcriptomic analysis made on Persian lime by our group (Estrella-Maldonado et al., *in preparation*). A search for sequences containing one or two Aspartic Protease Domain (DTGS or DSGT) was conducted using HMMER v3.3.2 software (Wheeler and Eddy, 2013) against the Pfam database (Accession: PF00026) (Mistry et al. 2020). All sequences containing the Aspartic Protease Domain were recovered and used in the present analysis.

Phylogeny of *CDR-like* sequences

A phylogenetic tree was constructed using *CDR* genes identified in our Persian lime transcriptome as well as sequences from different *CDR* genes obtained from *P. trifoliata* and *C. sinensis* genomes available from a public Citrus Genome Database (<https://www.citrusgenomedb.org/>). The Multiple sequence alignments of *CDR* genes from the three species were aligned by multiple alignment based on Muscle algorithm (gap open = 15; gap extend = 3) using BioEdit Software (Hall, 1999). A non-weighted Maximum Parsimony analysis was conducted using T.N.T. Software (Goloboff et al. 2008), to reconstruct the phylogenetic relationships among sequences, using a New Search Technology approach (ratchet + sectorial search), and a bootstrap (1000 repeats) method was applied to resample. A strict consensus tree was constructed with all the most parsimonious trees.

Similarity analysis among ClCDR, CsCDR and PtCDR proteins

To compare the similarity between *CDR* proteins from the three species, all genes recovered from the phylogenetic tree were translated to protein using the online tool ExPASy (<https://web.expasy.org/translate/>) and the largest ORF was selected for further analysis. Then an alignment was constructed with a ClustalW algorithm (gap open = 15; gap extend= 3) using BioEdit Software (Hall, 1999). Also, a genetic identity matrix was calculated among all *CDR*protein sequences based on *p-distance* and a UPGMA dendrogram was constructed with the calculated distances (bootstrap=1000 repeats), both analyzes were realized in MEGA V.11 Software (Tamura et al. 2021). The conserved motif structures of ClCDR, CsCDR and PtCDR proteins were analyzed using Multiple Expectation Maximization for Motif Elicitation (MEME) Software (<http://meme-suite.org/tools/meme>).

Expression patterns of *CICDR* genes in Persian lime during HLB infection

To determine the expression of *CICDR* genes, the differentially expressed genes (DEG's) database generated by Estrella-Maldonado et al. (*in preparation*) was consulted to obtain data of reads counts, Log₂ Fold change, and *p*-values of transcripts identified as *CDR* sequences.

CICDR expression validation by RT-PCR and qRT-PCR

Physiologically matured leaves (V5 stage according to scale proposed by Ribeiro et al. 2021) from 7 year-old Persian lime trees naturally infected by HLB, with and without symptoms were collected for RNA extraction. The CLas infection was diagnosed using nested PCR protocol developed by Lee et al. (2010). Total RNA was extracted from the same tissue using Trizol® Reagent protocol (Thermo Fischer Scientific) and treated with RQ1 RNase-free DNase (Promega) to remove genomic DNA contamination. The concentration and purity of RNA samples were examined by NanoDrop™ 1000 Spectrophotometer (Thermo Scientific NanoDrop Technologies, LLC, Wilmington, DE, USA) and the quality was evaluated by 1.5% agarose gel electrophoresis during 30 min at 80 V. The single strand cDNA was synthesized using M-MLV reverse transcriptase enzyme (Promega), according to the manufacturer instructions. A set of primers were designed using Primer Express Ver. 3.1 (Applied Biosystems USA) to evaluate the relative expression levels of *CICDR* genes.

First, gene expression was evaluated by RT-PCR using the amplification product of *SAND* gen as control (Mafra et al. 2012). The reaction condition was 10X PCR buffer, MgCl₂ 25 mM, dNTP's 10 mM, 10 µM of each forward and reverse primer, 1 U of Taq DNA polymerase (Invitrogen) and 600 ng/µl of cDNA, at a final volume of 25 µl. Products were analyzed in agarose gels (1.5 %, 60 V, 90 min), dyed with ethidium bromide (2%) and visualized under UV light. The relative expression levels of *CICDR8* gen was validated by qRT-PCR, and *UPL7* gen was used as reference to normalized all data (Mafra et al. 2012). The reaction specificity was confirmed using standard melt curve method. The mixture of each tube comprised the following constituents: 300 ng cDNA, 2X Sybr green master mix (Biorad), 5 µM of each primer and 3 µl of ultrapure water. The reaction was performed in a thermocycler StepOne™ Real-Time PCR Systems and StepOne Software v2.3 (Applied Biosystems, Foster City, CA, USA). Relative Expression Levels (REL) of *CICDR8* gene in symptomatic and asymptomatic leaves, was calculated using the 2^{-ΔΔCT} method (Schmittgen and Livak 2008). qRT-PCR conditions were as follows: 95 °C for 3 min; 40 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s. The melting curves were analyzed at 60 °C to 95 °C after 40 cycles. REL data were subjected to a bilateral T-Student analysis in R Software.

Results

Identification of CDR-like genes in *C. latifolia* transcriptome and phylogenetic homology

A total of 146 sequences from the Persian lime transcriptome, showed the aspartyl protease domain. These sequences were used for Maximum Parsimony phylogenetic reconstruction along with the *CDR* sequences from *P. trifoliata* and *C. sinensis*. The search allowed the recovery of five most parsimonious trees (L = 789), and a strict consensus tree was constructed. There was seven clades where at least one *PtCDR* or *CsCDR* gene sequence and at least a transcript from *C. latifolia* were grouped. The clade containing the *PtCDR1* also contain the transcript TRINITY_DN735_c2_g1_i1, the clade was well bootstrap supported; other clade grouped the *PtCDR8* gene with different isoforms of the transcript TRINITY_DN3834_c0_g1, this clade showed similarity with

the clade containing the *PtCDR3* which also have a isoform of the transcript TRINITY_DN3834_c0_g3_i1. On the other hand, the transcript TRINITY_DN9086_c0_g1_i1 showed high similarity with *PtCDR6* gene, however, the transcripts TRINITY_DN12502_c0_g2, TRINITY_DN18041_c0_g1_i2 and three isoforms of the TRINITY_DN735_c0_g1 transcript also showed similarity with *PtCDR6* gene of *P. trifoliata*. Finally, two clades were recovered with sequences from *C. sinensis*, the clade with *CsCDR3* which was homologous to the TRINITY_20694_c0_g1_i1 transcript, and the *CsCDR13*, homologous to four isoforms to the TRINITY_DN5173_c0_g1 transcript (Fig. 1) and all *CICDR* genes identified are summarized in Table 1.

Table 1
Identification of *CDR* genes in the *C. latifolia* transcriptome and homologous in *P. trifoliata* and *C. sinensis*.

Transcript sequence	<i>C. latifolia</i> <i>CDR</i> gene	Homologous
TRINITY_DN5173_c0_g1_i2	<i>CICDR3</i>	<i>CsCDR3</i>
TRINITY_DN12502_c0_g2_i1	<i>CICDR4</i>	-
TRINITY_DN735_c0_g1_i3	<i>CICDR5</i>	-
TRINITY_DN9086_c0_g1_i1	<i>CICDR6</i>	<i>PtCDR6-PtCDR1</i>
TRINITY_DN3834_c0	<i>CICDR8</i>	<i>PtCDR8-PtCDR3</i>
TRINITY_DN20694_c0_g1_i1	<i>CICDR13</i>	<i>CsCDR13</i>

Molecular Similarity Of Clcdr, Ptcdr And Cscdr Proteins

The amino acids sequences multiple alignment showed that there are some highly conserved regions, especially related to functional aspartyl protease domains (Fig. 2). However, the transcript TRINITY_DN18041_c0_g1_i1 did not show any of the two catalytic domains, therefore it was discarded for further analysis. All the other sequences showed DTGS domain, but the TRINITY_DN12502_c0_g2_i1 protein had a modified DSGS domain, also the TRINITY_DN2094_c0_g1_i1 protein had a modified DTSN domain. Interestingly, the *CsCDR13* protein had a modified DTAS domain. Regarding the DSGT domain, TRINITY_DN2094_c0_g1_i1 protein was the only one that did not present this domain, however, the sequences TRINITY_DN12502_c0_g2_i1 and TRINITY_DN735_c0_g1_i3 proteins, had the domain modified as DTGT and DCGT, respectively. The protein *CsCDR3* also showed the DSGS domain modified. The similarity matrix showed that some transcripts from Persian lime have, encode for proteins very similar to its homologous from *P. trifoliata* or *C. sinensis* with similarity values over 0.90. For instance, the transcript TRINITY_DN3834_c0_g3_i1 had 93.6% similarity value. Interestingly, the largest ORF encoded by the transcript TRINITY_DN9086_c0_g1_i1 was identical to the protein encoded by the *PtCDR1* gene (100%), however, it also showed high similarity with *PtCDR6* (99.6%) despite not showing phylogenetic homology among the transcript and the *PtCDR1* gene, suggesting that this protein have multiple origins in the *P. trifoliata* genome, but it is unique in *C. latifolia*. In addition, low similarity of *CDR* genes between *C. latifolia* and *C. sinensis* was observed (Table 2).

Table 2

Protein similarity matrix percentage among proteins codified from *CICDR* transcripts versus *PtCDR* and *CsCDR* proteins. Similarity values over 90% are marked on bold letter.

	PtCDR1	PtCDR3	PtCDR6	PtCDR8	CsCDR3	CsCDR13
TRINITY_DN3834_c0_g3_i1	28.6	93.6	28.4	80.8	30.7	41.7
TRINITY_DN9086_c0_g1_i1	100	28.0	99.6	29.8	32.9	30.4
TRINITY_DN12502_c0_g2_i1	50.4	31.6	50.0	29.9	29.3	27.9
TRINITY_DN735_c0_g1_i3	49.9	31.2	49.7	31.1	29.7	27.0
TRINITY_DN3834_c0_g1_i4	29.0	78.1	29.0	95.2	29.0	42.3
TRINITY_DN20694_c0_g1_i1	28.3	28.9	27.7	34.4	26.8	25.3
TRINITY_DN5173_c0_g1_i2	24.7	25.2	24.5	25.2	23.0	26.2

Respect to conserved motifs analysis, the phylogenetic homologous proteins, the motifs and its organization was very similar. For instance, the protein encoded by transcript TRINITY_DN9086_c0_g1_i1 had the same motifs in the same position and length that the ones observed in PtCDR1 and PtCDR6 proteins. PtCDR3 and PtCDR8 proteins had very similar patterns of conserved motifs with both isoforms of transcript TRINITY_DN3834_c0, according to the phylogeny and similarity matrix. Also, the proteins encoded by the transcripts TRINITY_DN12502_c0_g2_i1 and TRINITY_DN735_c0_g1_i3 had similar patterns to other CDR proteins, but the number and organization of them are different, suggesting that this are *CICDR* genes specific of *C. latifolia*. CsCDR3 and TRINITY_DN5173_c0_g1_i2 proteins share four conserved motifs, including the catalytic domains, supporting the homology between them. For CsCDR13 and TRINITY_DN20694_c0_g1_i1, the analysis showed that in the later, the motif containing the DTGS domain is not complete at the C-terminal (Fig. 3).

CICDR genes expression in the Persian lime transcriptome during HLB infection

Data obtained from the Persian lime HLB infected transcriptome, showed that the majority of the transcripts from *CICDR* genes had a low count, Log₂ fold change < 2, and a *p-value* > 0.05, indicating that there is no differences in *CICDR* genes expression in Persian lime during HLB infection (Table 3).

Table 3
Counts per transcript, Log₂ fold change and *p-value* associated to each *CICDR* gene transcript.

Transcript sequence	<i>C. latifolia</i> <i>CDR</i> gene	Transcriptome counts in asymptomatic*	Transcriptome counts in symptomatic*	Log ₂ fold change	<i>p-value</i>
TRINITY_DN5173_c0_g1_i2	<i>CICDR3</i>	174.6	234.6	0.071	0.84
TRINITY_DN12502_c0_g2_i1	<i>CICDR4</i>	9.0	12.3	0.079	0.97
TRINITY_DN735_c0_g1_i3	<i>CICDR5</i>	526.6	414.3	-0.073	0.12
TRINITY_DN9086_c0_g1_i1	<i>CICDR6</i>	37.0	57.0	0.28	0.64
TRINITY_DN3834_c0_g1	<i>CICDR8</i>	2030.6	1811.6	-0.45	0.45
TRINITY_DN3834_c0_g3	<i>CICDR8</i>	779.0	133.3	-1.25	0.06
TRINITY_DN20694_c0_g1_i1	<i>CICDR13</i>	10.6	6.0	-1.04	0.72

RT-PCR amplification of *CICDR* genes and qRT-PCR validation of *CICDR8* gene

We obtained DNA and RNA from three samples of Persian lime leaves with HLB symptoms and three samples without symptoms (corroborating that the asymptomatic samples were not infected with *CLas*; data not shown). Then, the cDNA was synthesized and the amplification of each *CICDR* was performed and visualized in agarose gel. No amplification was observed in almost all *CICDR* genes, except in the case of *CICDR8a* (TRINITY_DN3834_c0_g1 transcript). The amplification pattern observed in the electrophoresis corresponds with the number of counts in the transcriptome data, suggesting that the number of mRNA of *CICDR* genes in the samples, (symptomatic or asymptomatic), was so low that it was not possible its qualitative observation through the RT-PCR method, unlike the *CICDRa* and the control *SAND* gene (Fig. 4a). We compare the REL between HLB symptomatic and asymptomatic Persian lime samples using qRT-PCR of the *CICDR8a* gene, the results of the statistical analysis showed that there is no significant differences in the expression of this gene when the HLB infection is established, concordantly with the transcriptome DEG analysis (Fig. 4b).

Discussion

HLB disease is a major concern for many farmers, technicians and researchers around the world. Due to the difficulty of *CLas* isolation (Merfa et al. 2019), the development of strategies to directly attack the bacteria causing the disease have been rather slow, making the generation of genetic resistance to the pathogen the most promissory tool for long time-management of HLB (Martinelli and Dandekar, 2017; Li et al. 2021). Hence, the study of different genotypes with different levels of HLB-tolerance is very important to understand the molecular mechanisms of this tolerance and then use it in breeding programs aiming the generation of HLB-tolerant genotypes.

One of the most tolerant citrus species is *P. trifoliata* (Albrecht and Bowman, 2012), and it has been suggested that the overexpression of the *CDR* gene family in response to the disease, may be an important part of the molecular mechanism associated to its superior HLB-tolerance. On the contrary, very susceptible genotypes showed low *CDR* expression in response to the disease (Rawat et al. 2017). Our results show that there was at least six *CDR* genes present in the transcriptome of Persian lime, two phylogenetically homologous to *PtCDR*

genes and two homologous to *CsCDR* genes. Interestingly, the protein encoded by transcript TRINITY_DN9086_c0_g1_i1 (*CICDR6*) is very similar to two proteins from *P. trifoliata*, *PtCDR1* and *PtCDR6*, but the gene is homologous to *PtCDR6* not to *PtCDR1*, this results is concordant with Rawat et al. (2017), which associated both *PtCDR* genes with just one gene from *C. sinensis*; the position of both genes in the phylogeny suggest that in *P. trifoliata* this protein have multiple origins with a convergent evolution (Storz, 2016), unlike to *C. sinensis* and *C. latifolia* with just one orthologous in their genomes.

On the contrary, the gen *CICDR8* translate at least two different isoforms that are phylogenetically homologous to *PtCDR8* and *PtCDR3* genes, and encode proteins very similar to *PtCDR8* and *PtCDR3* proteins; moreover, they are homologous to *PtCDR2* gene. Recently, heterologous expression of *PtCDR2* and *PtCDR8* genes in *Arabidopsis cdr1* mutant demonstrated the ability to restore the hypersensitive response (HR) to *Pseudomonas syringae*, associating the HR with high expression levels of PR genes (Ying et al. 2020), supporting the hypothesis that the overexpression of this genes are associated to HLB tolerance. In *C. latifolia* a homologous to *PtCDR2* was not observed, but it was found a homologous to *PtCDR8*; contrary to the expected results, the HLB infection did not induce a detectable overexpression of this gene (*CICDR8*), neither in transcriptome or qRT-PCR results, suggesting that this gene is not related to the tolerance observed in this citrus specie (Sivager et al. 2021), although the basal expression of this gene is high in healthy plants. Also, this work is the first evolutionary approximation to the complexity of *CDR* family, and evidence suggest that there are many copies of genes encoding similar proteins in *P. trifoliata* (*PtCDR2*, *PtCDR3* and *PtCDR8*), with tentatively redundant functions, while that in *C. latifolia* it is a unique gene encoding different proteins by alternative splicing, as it has been reported in others citrus crops (Del Carratore et al. 2011; Wu et al. 2021). In addition, the low counts number in the transcriptome, and the no amplification by RT-PCR of all the others *CICDR* genes, including the *CICDR8* isoform homologous to *PtCDR3* gene, are consistent with the behavior of this family in others citrus plants such as *C. sinensis* (Rawat et al. 2017). Those results are evidence that HLB does not induce the overexpression of this family in *C. latifolia*, that is, the tolerance reported in Persian lime is not associated to *CDR* family.

The results shown in the present work demonstrate that there are at least six members of the *CDR* family in *C. latifolia*, some of them are homologous to *PtCDR* and *CsCDR* genes. However, the tolerance reported previously in *C. latifolia* against HLB, is not associated with the overexpression of this gene family. Further studies are indeed necessary to elucidate which other gene families may be overexpressed in response to the disease that may explain the relatively high HLB-tolerance shown by *Citrus latifolia*.

Declarations

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Author Contributions

FRFR, GCC, designed and conducted the experiment. **AGJ, CLA**, qRT-PCR assays and helped to write manuscript. **SMR, JMS**, assisted in qRT-PCR assays and final manuscript writing. **EMH**, corresponding author, formal analysis and helped to write final manuscript. All authors read, commented on and approved the final manuscript.

Conflicts of Interest

The authors have no interest conflicts to declare that are relevant to the content of this article.

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Figures

PtCDR1	GFSSSVISXLAQGSGEYFTRLGVGTTPPRYVYVMVIDTGS	OVVWIIQCA	XCK
PtCDR3	SKVSQADIIPNVG--XYLIRISIGTTPPEILAVADTGS	OLIWTQC	QPCP
TRINITY DN3834 c0 g3 i1	SKVSQADIIPNVG--DYLIRISIGTTPPEILAVADTGS	OLIWTQC	QPCP
PtCDR6	GFSSSVISXLAQGSGEYFTRLGVGTTPPRYVYVMVIDTGS	OVVWIIQCA	PCK
TRINITY DN9086 c0 g1 i1	GFSSSVISGLAQGSGEYFTRLGVGTTPPRYVYVMVIDTGS	OVVWIIQCA	PCK
TRINITY DN12502 c0 g2 i1	DFGTDVVSQMDQGSGEYFVRIGVGSPPRSQYMV	DSGS	DIVWVQCQPCS
TRINITY DN18041 c0 g1 i1	-----	-----	-----
TRINITY DN735 c0 g1 i3	DFSTPVSQGSQGSGEYFSRIGVGTTPPRQFSMVIDTGS	DINWLQC	RPCT
PtCDR8	SKASQADIIPNIG--DYLIRISIGTTPATEILAVADTGS	OLIWTQC	CEPCP
TRINITY DN3834 c0 g1 i4	SKASQADIIPNNA--NYLIRISIGTTPETERLAVADTGS	OLIWTQC	CEPCP
CsCDR3	IIDYQADVFPKVSFLFFMNFITIGQPPIPQFTVM	DTGS	LLWVQCRPCL
TRINITY DN20694 c0 g1 i1	-----MAM	DTSNDA	AWVPCTGCV
CsCDR13	PEALQAPMVSSGG--EYFVNISIGTTPRVELIGIA	DTAS	DLTWIQCKPCY
TRINITY DN5173 c0 g1 i2	RMRLYDDLLSNG---YYTTRLNIGTTPQQFALIV	DTGS	TVTYVPCSTCQ

PtCDR1	ITASLFLKLDPAAGNGGVII	DSGT	SVTRLTRPAYIALRDAFRAGASS
PtCDR3	VISG-----SNPGGDIV	DSGT	TLTYLPPAYASKLLSVMSSMIAA
TRINITY DN3834 c0 g3 i1	VISG-----STPGGDIV	DSGT	TLTYLPPAYASTLLSVMSSMIAA
PtCDR6	ITASLFLKLDPAAGNGGVII	DSGT	SVTRLTRPAYIALRDAFRAGASS
TRINITY DN9086 c0 g1 i1	ITASLFLKLDPAAGNGGVII	DSGT	SVTRLTRPAYIALRDAFRAGASS
TRINITY DN12502 c0 g2 i1	ISEDLFRLTQMGGDDGV	MDTGT	AVTRLPTTAYEEFRDAFVAQTGN
TRINITY DN18041 c0 g1 i1	-----	-----	-----
TRINITY DN735 c0 g1 i3	IPPSLFEMDEAGDGGIIV	DCGT	AITRLQTQAYNSLRDSFVRLAGN
PtCDR8	VMSG-----STPGGDIV	DSGT	TLTLLPQXYNSKLLSVMSSMIEA
TRINITY DN3834 c0 g1 i4	VMSG-----STPGGGIV	DSGT	TLTLLPQGYNSKLLSVMSSMIEA
CsCDR3	IDPDI	FTRKTWDNGGVII	DSGSSATWLVKAGYDALLHEVESLLDM
TRINITY DN20694 c0 g1 i1	-----	-----	-----
CsCDR13	YFTSNAT--DGEENMI	DSGT	TFTFLDPQFYEKLA
TRINITY DN5173 c0 g1 i2	VSPR-IF--DGGHG-TV	DSGT	TYAYLPGHAF

Figure 2

Partial alignment of CDR proteins from *C. latifolia*, *C. sinensis* and *P. trifoliata*. The two catalytic domains are delimited.

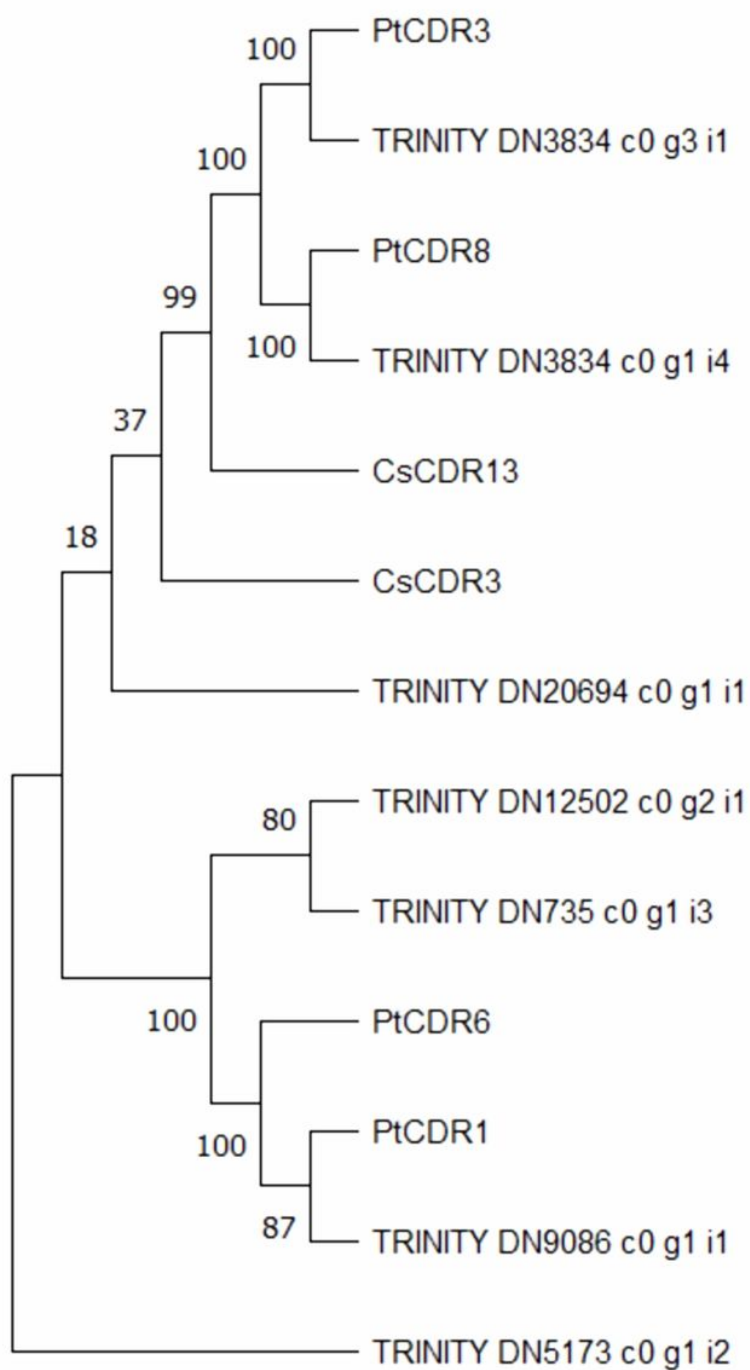


Figure 3

UPGMA dendrogram constructed based in the *p-distances* of CDR protein sequence from *C. latifolia*, *C. sinensis* and *P. trifoliata*.

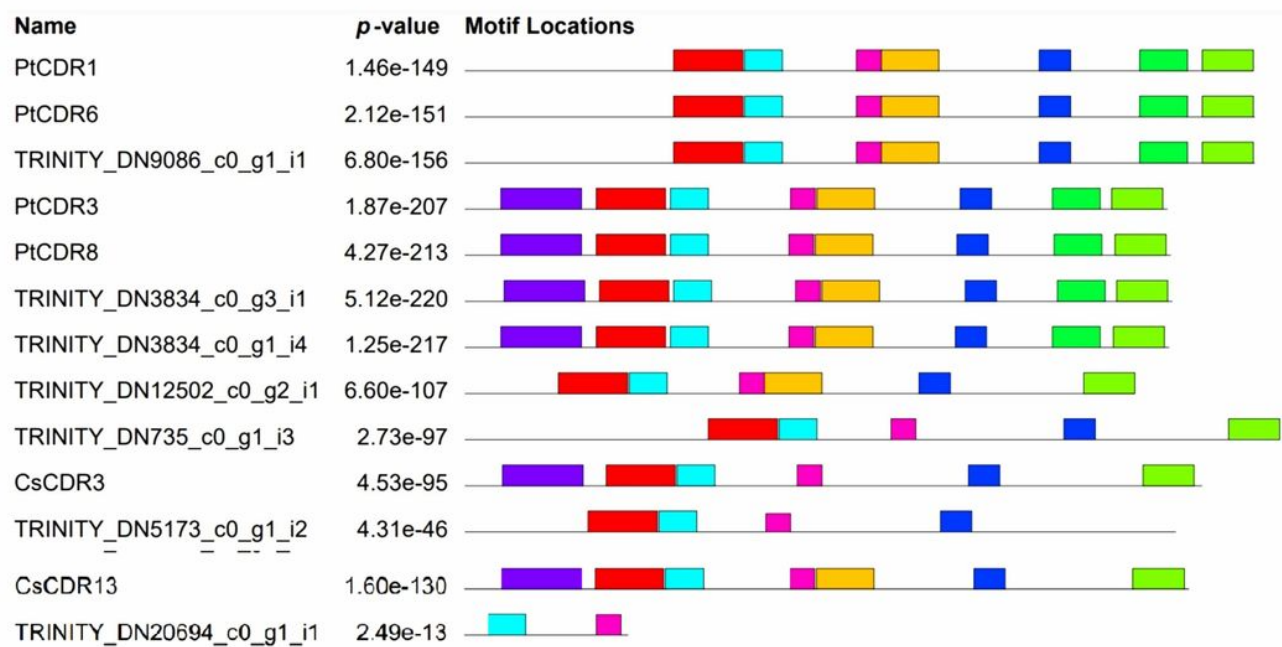


Figure 4

Conserved motifs search and location in the CDR protein sequences from *C. latifolia*, *C. sinensis* and *P. trifoliata*.

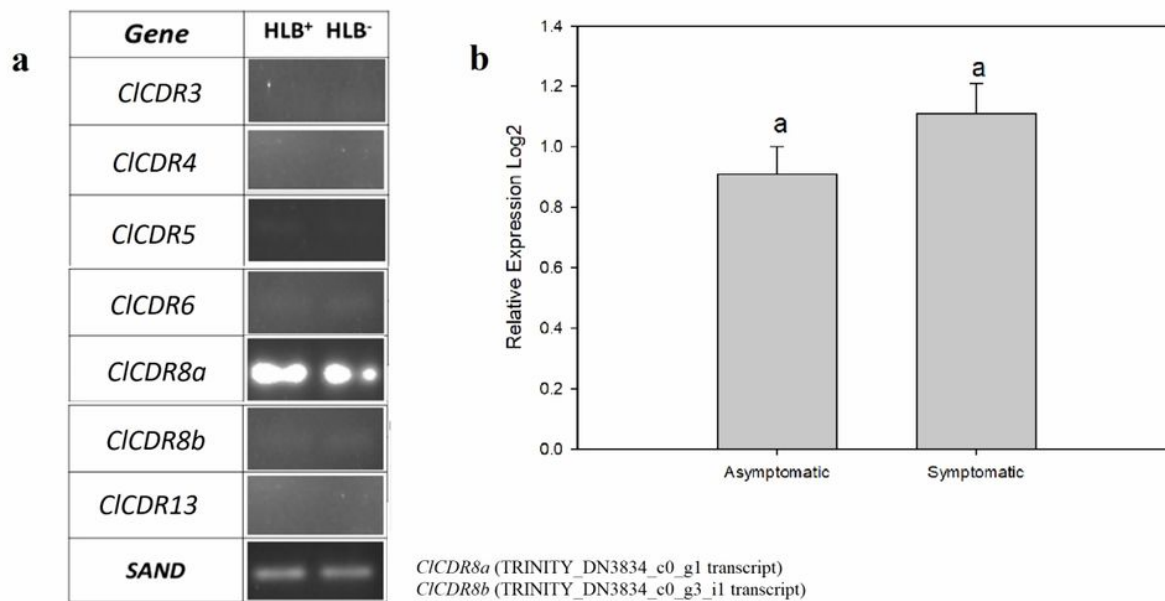


Figure 5

Amplification of *CICDR* genes from leaves of Persian lime with HLB symptoms (HLB⁺) and asymptomatic (HLB⁻), obtained from Persian lime transcriptome. **a)** Expression of *CICDR* genes by RT-PCR. **b)** Relative expression levels of *CICDRa* gene by qRT-PCR.