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ORIGINAL ARTICLE

Disturbance of *Arabidopsis thaliana* microRNA-regulated pathways by *Xcc* bacterial effector proteins

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Abstract Plants are continuously subjected to infection by pathogens, including bacteria and viruses. Bacteria can inject a variety of effector proteins into the host to reprogram host defense mechanism. It is known that microRNAs participate in plant disease resistance to bacterial pathogens and previous studies have suggested that some bacterial effectors have evolved to disturb the host's microRNA-regulated pathways; and so enabling infection. In this study, the interspecies interaction between an *Xanthomonas campestris pv campestris* (*Xcc*) pathogen effector and *Arabidopsis thaliana* microRNA transcription promoter was investigated using three methods: (1) interolog, (2) alignment based on using transcription factor binding site profile matrix, and (3) the web-based binding site prediction tool, PATSER. Furthermore, we integrated another two data sets from our previous study into the present web-based system. These are (1) microRNA target genes and their downstream effects mediated by protein–protein interaction (PPI), and (2) the *Xcc*–*Arabidopsis* PPI information. This present work is probably the first comprehensive study of constructing pathways that comprises effector, microRNA, target genes and PPI for the study of pathogen–host interactions. It is expected that this study may help to elucidate the role of pathogen–host interplay in a plant's immune system. The database is freely accessible at: http://ppi.bioinfo.asia.edu.tw/EDMRP.

Keywords *Arabidopsis thaliana* · Xanthomonas campestris pv campestris · Bacterial effector protein · Protein–protein interaction · Pathogen–host interactions · Transcription factor binding site

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Introduction

Pathogen-associated molecular patterns (PAMPs) trigger plant defenses when perceived by surface-localized immune receptors. PAMP-triggered immunity (PTI) plays an important role in the resistance of plants to pathogens. Many plant pathogens, including bacteria and viruses, can deliver a variety of effector proteins into the host plant cell to inhibit PTI signaling (Gohre and Robatzek 2008; Zhou and Chai 2008). In response, plant resistance proteins sense effectors to activate effector-triggered immunity (ETI), which is a second inducible defense layer (Jones and Dangl 2006; Chisholm et al. 2006).

Many gram-negative bacteria pathogens use type III secretion systems to inject proteins directly in to the cells of their hosts (Galan and Wolf-Watz 2006) which then manipulate host cellular processes to promote infection (Oh and Beer 2005; Cunnac et al. 2009; Kay and Bonas 2009; Poueymiro and Genin 2009). A type III secretion system comprises three components, i.e., effector, chaperone and nanoinjector. The injection of effector proteins into host cells can potentially block many plant cellular biological processes; such as transcription, cell death, plant

innate immunity, hormone pathways, and chloroplast function (Gohre and Robatzek 2008; Zhou and Chai 2008; Block et al. 2008; Speth et al. 2007).

Xanthomonas campestris pv campestris (*Xcc*) is one of the pathogenic bacteria that are known to cause diseases such as blights and rots in plants (Tsuji and Somerville 1988, 1992; Tsuji et al. 1991; Buell 2002). In addition, Xcc is considered the most important and most destructive disease of crucifers, infecting all cultivated varieties of brassicas world wide. Host infection by *Xcc* can occur at any stage of the plant life cycle.

It is well-known that *Arabidopsis thaliana*, a long-day plant, is a good model organism for plant science (Mandoli and Olmstead 2000). *A. thaliana* is chosen as the model system for two reasons: (1) the complete genome sequence has been known since 2000; and (2) there are many molecular tools, such as cDNA, genomic libraries, bacterial artificial chromosomes, microarrays, and ESTs which are available for the study of biological functions (Mandoli and Olmstead 2000).

In plants, small RNAs including microRNA and small interfering RNAs (siRNAs) are involved in antiviral defenses (Mourrain et al. 2000; Dalmay et al. 2001; Morel et al. 2002). Several studies have indicated that small RNAs also participate in plant disease resistance to bacterial pathogens. For instance, flg22 induces the accumulation of miR393, which contributes to plant resistance against bacteria by negatively regulating the mRNA level of F-box auxin receptors TIR1, AFB2, and AFB3 (Navarro et al. 2006).

The work of Navarro et al. (2008) has indicated that *A. thaliana* microRNA-deficient mutants partly restore growth of type III *Pseudomonas syringae* mutants, these mutants also sustained growth of nonpathogenic *Pseudomonas fluorescens* and *Escherichia coli* strains, implicating microRNA as a key component of plant basal defense. These results provide evidence that, like viruses, bacteria have evolved to suppress RNA silencing. In addition, there is some evidence that the *A. thaliana* miR393 is PAMP-responsive (Navarro et al. 2008; Li et al. 2010) and contributes to resistance against virulent *Pseudomonas syringae pv. Tomato* strain DC3003 (Navarro et al. 2008). But the full extent to which microRNAs participate in PAMP-triggered immunity (PTI) in plants remains unknown.

The idea underlying our work is to identify *Xcc* effector binding sites on *A. thaliana* microRNA promoter regions and to study the affect of the *Xcc* effector on *A. thaliana* microRNA-regulated pathways. In this study, inter-species protein–DNA interaction (PDI) between *Xcc* effector protein and *A. thaliana* microRNA was predicted by two different approaches, which are: (1) interolog and (2) alignment based on transcription factor binding site (TFBS) profile matrix. It is also important to identify microRNA target genes in the host system, and study their downstream effects. Therefore, we incorporated the target genes information along with the microRNA-related protein–protein interaction (PPI) pathways from our previous study (Kurubanjerdjit et al. 2012). Pathogen proteins can disturb the host system at the PPI level, hence, the *Xcc–Arabidopsis* PPI information was also integrated into the present web-based platform using our previous results. In summary, a unified dataset comprising effectors, microRNAs, target genes and PPI was established for pathogen–host interaction study. Such a system on an effector-disturbed microRNA-regulated pathway will be useful for revealing the overall biological pathways of a plant's immune system.

Materials and methods

Data sources

To implement the interolog approach, a collection of 202 *Xcc* protein sequences were downloaded from UniProt, and a set of 187 *A. thaliana* microRNA promoter sequences (3 kb upstream of the microRNA transcription region) was obtained from the Plant MicroRNA Database (PMRD) (Zhang 2010). Experimentally confirmed PDIs were gathered from Protein-DNA Interface Database (PDIdb: http://melolab.org/pdidb/web/content/home) (Norambuena and Melo 2010). This online database is a repository containing relevant structural information of protein–DNA complexes resolved by X-ray crystallography and available at the Protein Data Bank (PDB).

To perform pathogen–host PDI prediction, we proposed to align the microRNA promoter sequence against the TFBS profile matrix. A total of 164 TFBS profile matrices from a variety of prokaryotic transcription factors (TF) were retrieved from PePPER (de Jong et al. 2012), and their protein sequences were obtained from UniProt. In the present study, the interaction between *Xcc* effector and *A. thaliana* microRNA were identified using two pipelines as shown in system flowchart in Fig. 1. Firstly, potential PDIs were predicted using the interolog approach and then the predicted PDI of *Xcc* effector and *A. thaliana* microRNA promoter was identified using the TFBS profile matrix of *Xcc* homolog proteins.

In addition, three different types of interactions were identified and integrated to examine the interaction between *A. thaliana* and *Xcc*, which are (1) binding of *Xcc* effectors to *A. thaliana* microRNA promoter regions; (2) the interaction of *A. thaliana* microRNA and their target gene; and (3) the PPI of *A. thaliana* and *Xcc* proteins. Figure 2 shows the integrated system for constructing the pathogen–host interaction pathways.

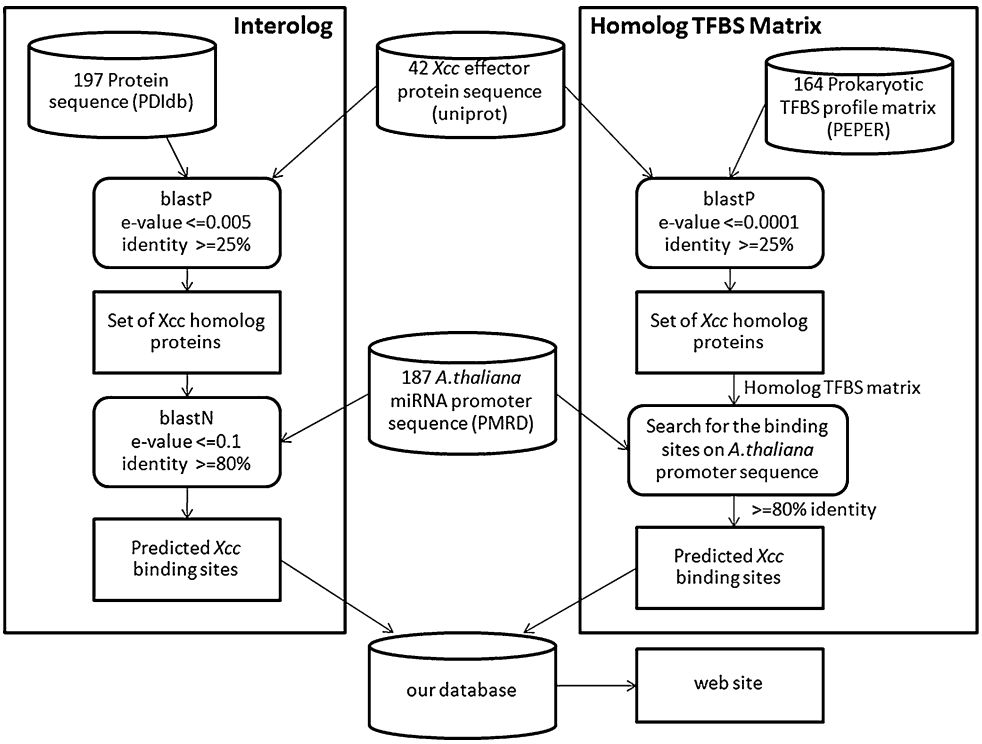

Fig. 1 System flowchart of PDI prediction between *Xcc* effector and *A. thaliana* microRNA. Prediction was based on the (1) interolog and, (2) alignment of homolog TFBS profile matrix approaches

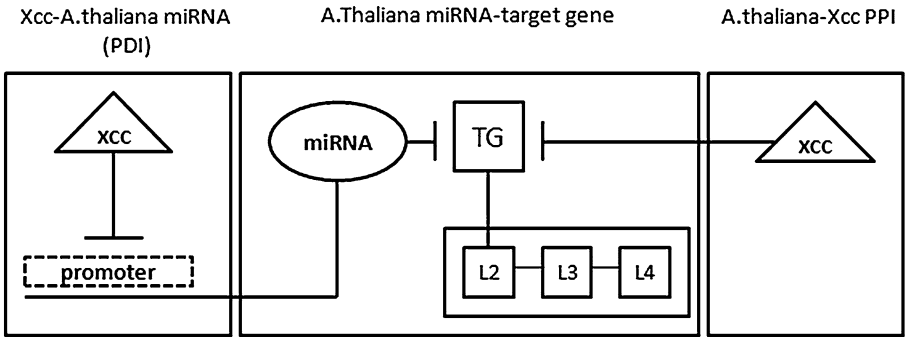

Fig. 2 The pathogen–host integrating pathways comprises bacterial effector (*triangle*), promoter (*dotted line rectangle*), microRNA (*ellipse*), target gene (TG, *square*), and protein (*square*, L2, L3 and L4 denote the second, third and fourth level of PPI)

Xcc effector protein prediction

To identify the *Xcc* effector protein, two efficient prediction tools were employed; Effective T3 (http://www.effectors.org/) (Jehl et al. 2011) and the type III secretion system effector prediction system developed by the Molecular design laboratory (ModLab, http://gecco.org.chemie.uni-frankfurt.de/index.html).

Prediction of protein–DNA interaction by interolog approach

We adopted BLAST to search for *Xcc* effector binding sites on *A. thaliana* promoter regions. The lower the e-value, or the closer it is to zero, the more significant the match is. Short alignments have relatively high e-values, this is because the calculation of the e-value takes into account the length of the query sequence. In general, one sets the e-value to be 0.0001 for blastP and blastN search (Claverie and Notredame 2006). Protein sequences obtained from PDIdb have an average length of 116 bases, whereas an average length for homologous TFBS study have an average of 404 bases; therefore, a relatively high e-value, i.e., 0.005 is chosen for the interolog study. An e-value of 0.1 is chosen for the blastN search, because the average binding DNA sequence length from PDIdb is 15 bases.

BlastP was used to search for homologous *Xcc* effectors. Forty-two predicted effector proteins were blasted against 197 proteins of various species obtained from PDIdb with the e-value cutoff set to 0.005. A filter was applied to this set of *Xcc* homolog proteins to ensure greater than 25 % sequence identity. The interacting nucleotide sequences of the *Xcc* homolog proteins were then adopted as input to perform blastN, and searched against 187 *A. thaliana*

microRNA promoter sequences, with an e-value cutoff setting of 0.1. Finally, *A. thaliana* microRNA promoter regions with more than 80 % sequence identity were identified as *Xcc* effector binding regions.

Prediction of protein–DNA interaction by aligning sequence against TFBS profile matrix

Firstly, the 42 predicted *Xcc* effector proteins were blasted against prokaryotic TFs with the e-value cutoff set to 0.0001. This alignment allowed us to identify *Xcc* homolog TFs. Then this set of *Xcc* homolog TF proteins was filtered to enforce a sequence identity of greater than 25 %. Secondly, the *Xcc* effector binding site along *A. thaliana* microRNA promoter sequences was identified using a sliding TFBS matrix window approach and PATSER tool.

TFBS matrix sliding-window approach

By implementing this approach, the TFBS matrix consists of four rows of the weighting A, C, G, T occurrence scores in each position represented in column, the sliding window size is equal to the length of the TFBS matrix. The matching scores are calculated by sliding a TFBS matrix window across aligned promoter sequences 1 bp at a time, a window is applied at each region in order to sum up the score of hits that are presented in the window of all positions. Thus, the region which gives the highest matching score of each microRNA promoter sequence is assigned as the best binding region. Finally, only the binding regions which satisfied the 80 % identity bound were selected. The identity measure is computed based on Eq. 1, where α denotes the matching score of the binding region and β denotes the maximum matching score of the TFBS profile matrix.

$$\text{Identity} = \frac{\alpha}{\beta} \quad (1)$$

PATSER tool

RSA-Tools-PATSER was adopted in this study to analyze the matching positions on microRNA promoter sequence according to TFBS matrix profiles. PATSER was developed by Stormo Lab at Washington University. This tool can predict putative matching positions by scanning a DNA sequence with a position-specific scoring matrix (PSSM). The same set of 42 predicted *Xcc* effector proteins and TFBS profile matrixes were submitted to PATSER (http://rsat.ulb.ac.be/patser_form.cgi). Default parameters are used for TFBS prediction, for instance, options "all matches" and "weight score = 7" are adopted. Binding sites with relatively small weight scores are likely to be false predictions. Finally, a set of binding sites in which the score was greater than seven was obtained.

Prediction of TFBS on microRNA host gene promoter region

We predicted TFBS on promoter regions of microRNA host genes based on the assumption that if TFBS has been found along microRNA promoter sequences, it is expected that such a region may be the binding site of a pathogen effector. *A. thaliana* TFBS motif information was obtained from *A. thaliana* Promoter Binding element Database (AtProbe: http://exon.cshl.org/cgi-bin/atprobe/atprobe.pl) and *Arabidopsis* Gene Regulatory Information Server (AGRIS: http://arabidopsis.med.ohio-state.edu/), and then we searched for the regions along microRNA promoter sequences which matched TFBS motifs.

Gene set enrichment analysis (GSEA)

The functional annotation of microRNA target genes where the promoter region is predicted to have PDI with the *Xcc* effector is given by implementing The Database for Annotation, Visualization and Integrated Discovery, i.e., DAVID (Huang da et al. 2009). DAVID provides functional annotation tools which accept batch annotation and conduct Gene Ontology (GO) term enrichment analysis. A list of microRNA targets (TAIR ID), which is potentially regulated by the *Xcc* effectors, was submitted to DAVID for clustering of the redundant annotation terms. Thus, enriched biological processes related gene lists were obtained.

The prediction of *A. thaliana* microRNA–Xcc pathogen pathways

To explore the effects of *Xcc* effector on *A. thaliana*, we integrated some results derived from our earlier study (Kurubanjerdjit et al. 2012). Firstly, the microRNA-regulated PPI pathways in *A. thaliana* are considered. MicroRNA targets are predicted by implementing various machine-learning classifiers. Then, the PPI partner information for the target genes was obtained from the BioGrid database. In particular, our system also provides plant resistance genes (PRG) information, obtained from PRGdb: Plant Resistance Genes Database (Sanseverino 2010), and TF annotations for the target genes.

Secondly, we derived the pathogen–host interaction pathway, i.e., *Xcc* and *A. thaliana* PPI pathway. In a previous study (Li et al. 2011), host–pathogen PPIs between bacterium *R. solanacearum* and *A. thaliana* were predicted based on the interolog and domain-based approaches. Again, blastP was used in the present analysis, the e-value cutoff are set to 10^{-70} and 10^{-4} (Yu et al. 2004; Li et al. 2011) for the interolog and domain-based approach, respectively. The results of these two predictions were

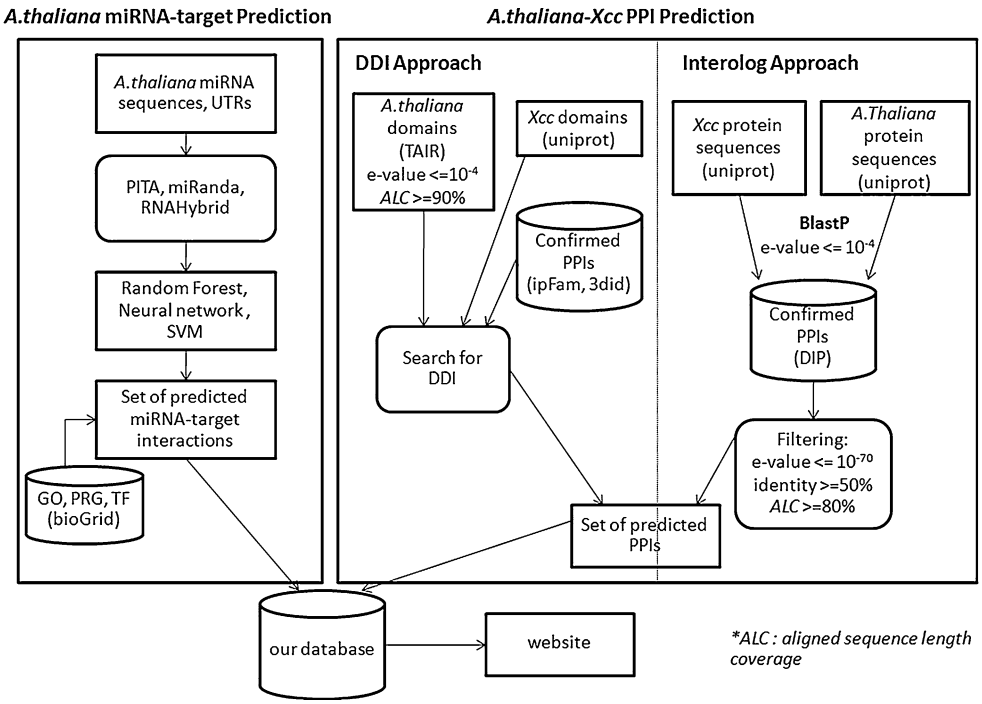

Fig. 3 System flowchart of *A. thaliana* microRNA–Xcc pathogen pathways prediction

integrated into the current system. Figure 3 shows the system flowchart of these two methods.

Results

Protein–DNA interaction prediction

Forty-two *Xcc* effector proteins were identified by EffectiveT3 and the ModLab tool. A total of 3,988 putative effector-binding sites on *A. thaliana* microRNA promoters were predicted, which involved three effectors and 187 microRNAs. Nine, 3,368 and 611 predicted binding sites were derived from the interolog approach, alignment of homolog TFBS profile matrix by sliding window and by PATSER. Two hundred binding sites were predicted by both the TFBS profile matrix and PATSER.

Our result indicates that the *Xcc* effector, P22260 (clp), which was predicted to bind to many microRNA promoter regions, is the type III effector protein involved in the pathogenesis process. This evidence is also consistent with the record in Uniprot, which indicates that this effector can enhance the ability of an organism to cause disease in another. Table 1 shows the domain composition and biological processes of the predicted *Xcc* effectors binding to *A. thaliana* microRNA promoter regions. It is interesting to note that the *clp* effector is composed of a DNA-binding domain, hence providing further evidence in support of the prediction.

Table 1 Domain composition and biological process of effectors bind to microRNA promoter regions

Xcc effector	PFam domain name	Biological processes
P22260 (*clp*)	Cyclic nucleotide-binding domain, Crp domain	Pathogenesis, transcription, DNA-dependent
Q8P8F9 (*apt*)	Phosphoribosyl transferase domain	AMP salvage, adenine salvage, purine ribonucleoside salvage
Q8PC11 (*cysA*)	ABC transporter, TOBE-like domain	ATP catabolic process, ATP-binding cassette (ABC) transporter complex, plasma membrane

Enriched biological processes of microRNA target genes

The over-represented biological processes of microRNA target genes where the microRNA is predicted to have PDI with *Xcc* effector are mainly enriched in (1) response to cadmium ions, (2) response to metal ions, and (3) in response to inorganic substances. Table 2 shows the top three biological processes for the microRNA target genes according to their e-value.

Previous studies have suggested that metal ions are required for pathogen virulence and plant defenses, i.e., high levels of Fe are needed for the infection process of bacteria (Franza et al. 2005), a high Zn or Mn is important in the mandibles of seed-penetrating larvae (Morgan et al. 2002), high level of Zn is required for an invasion of the

Table 2 Biological Processes of microRNA target genes

Xcc effector	Biological processes	*P* value
P22260	GO:0046686—response to cadmium ion	9.2×10^{-3}
	GO:0010038—response to metal ion	1.43×10^{-2}
	GO:0010035—response to inorganic substance	3.49×10^{-2}
Q8P8F9	GO:0046686—response to cadmium ion	5.26×10^{-2}
	GO:0010038—response to metal ion	6.99×10^{-2}
	GO:0010035—response to inorganic substance	1.25×10^{-1}
Q8PC11	GO:0046686—response to cadmium ion	9.2×10^{-3}
	GO:0010038—response to metal ion	1.43×10^{-2}
	GO:0010035—response to inorganic substance	3.49×10^{-2}

rice blast fungus (Tucker et al. 2004), Cu and Zn are major factors for superoxide dismutase in necrotrophic fungal pathogens and in plant defense against oxidative stress (Babitha et al. 2002; Rolke et al. 2004), and the regulation Zn homeostasis in bacteria is required for pathogen virulence (Tang et al. 2005).

Our result is consistent with the report by Stahl and Bishop (2000) in which plants resist a pathogen by using their defense response, i.e., proteins that function in respond to environmental stimulation are enriched. Our findings also support the work of Fones et al. (2010) who demonstrated that Zn, Ni, or Cd are accumulated when *Thlaspi caerule* resists leaf spot caused by *Pseudomonas syringae pv. maculicola* (*Psm*). They grew *Thlaspi* in soil with higher concentration of Zn, Ni and Cd, it was found that the degree concentration of these metals has a direct effect on the intensity of defensive mechanism of *Thlaspi*. Furthermore, the work by Banjerdkit et al. (2005) investigated the effects of Cd exposure on the oxidative stress responses of *Xcc* bacterium. Thus, *Xcc* uses this strategy to survive in the presence of reactive oxygen species.

Xcc effector binding site on *A. thaliana* microRNA promoter regions

From our results, there are multiple regions along the *A. thaliana* microRNA promoters that were predicted as *Xcc* effector binding sites. List of the P22260 and Q8P8F9 effectors' binding sites, which satisfied 90 % sequence identity bound is shown in Table 3. Three of the microRNAs—ath-MIR-408, ath-MIR-169i, and ath-MIR-169j—are upstream regulators of two PRG.

Cis-regulatory binding sites

The locations of cis-regulatory binding sites determine the connectivity of genetic regulatory networks. Identification

Table 3 Predicted effector-binding sites with identity of over 90 %

Xcc effector	MicroRNA promoter	Binding site	% of identity
P22260	ath-MIR165a	TTGACGGTCATCAA	100
	ath-MIR408	TTGATGTGGGTCAA	92.98
	ath-MIR472	TTGATCTTAATCAA	90.90
	ath-MIR782	TTGACAACGATCAA	90.90
	ath-MIR859	TTCACTTTCATCAA	90.90
	ath-MIR868	TATGTAATCTGAATCACATT	90.75
Q8P8F9	ath-MIR169i	TGGAAACGCTTACA	92.30
	ath-MIR169j	TGGAAACGCTTACA	92.30
	ath-MIR166a	TAAAAACGTTTTCA	90.76

Table 4 Predicted cis-regulatory binding sites along microRNA promoters

MiRNA targeted by *Xcc*	# Predicted Tfbs (sliding window)	# Predicted TFBS (PATSER)	# Total
ath-MIR856	65	4	69
P22260	4	2	6
Q8P8F9	2	2	4
Q8PC11	59	0	59
ath-MIR169l	48	4	52
P22260	3	3	6
Q8P8F9	1	1	2
Q8PC11	44	0	44
ath-MIR169k	46	4	50
P22260	5	3	8
Q8P8F9	1	1	2
Q8PC11	40	0	40

of these binding sites would facilitate research in certain key areas, including evolution, development and the pathogenic immunity system. Cis-regulatory binding sites on microRNA promoters where a number of transcription factors can bind were observed in this study. Table 4 shows the top three total numbers of binding sites predicted by both of TFBS approach and PATSER. Our finding revealed that ath-MIR169l has a total of 52 binding sites; of which 48 and four are predicted by the TFBS approach and PATSER, respectively. P22260, Q8P8F9 and Q8PC11 effector proteins can bind to the ath-MIR169l promoter region at six, two and 44 sites, respectively. It was interesting to note that ath-MIR169l targets a PRG, AT5g22330 (RIN1). In contrast to the effectors P22260 and Q8P8F9, Q8PC11 has many more binding sites predicted by the sliding-window approach. This is because the size of the profile matrix for Q8PC11 is seven, whereas P22260 and Q8P8F8 profile matrices have a size of at least 14. Null

results were obtained for PATSER because the prediction does not pass the weight score threshold.

Sliding window and PATSER results

Our results indicate that a set of microRNAs, ath-MIR164c, ath-MIR167a, ath-MIR167b, ath-MIR169i, ath-MIR169j, ath-MIR169k, ath-MIR169l, ath-MIR408, ath-MIR771, ath-MIR843 and ath-MIR854a, target the *PRG*, *RIN1*. This gene has been reported by TAIR (Rhee et al. 2003) to function in regulation of defense responses to fungi, incompatible interactions, and regulation of flower development. Furthermore, our findings suggested that five microRNAs, ath-MIR156g, ath-MIR168a, ath-MIR395c, ath-MIR395f and ath-miR447b, bind to a PRG, AT3G48750 (*CDC2*). Table 5 summarizes the binding sites sequence information predicted by both the sliding-window approach and PATSER for effectors P22260 and Q8P8F9. It was found that ath-MIR408 which targets *RIN1*, has four binding sites for P22260.

Web site

A web site has been set up to provide comprehensive information that comprises effector, microRNA, target genes and PPI for the study of pathogen–host interactions. The importance of this web site can be understood in terms of the following three pieces of information: (1) predicted interaction between pathogen effector and the host's microRNA promoter binding sites; (2) putative regulation relationship between microRNA and target genes, as well as the microRNA-regulated PPI pathways; and (3) the pathogen–host PPI information. As many studies have suggested, microRNA pathways play an important role in antibacterial defense, and we have identified effectors that may induce microRNA silencing. This result provides a starting point for postulating defense mechanisms. For point (2), if the upstream

Table 5 Effectors bind on microRNA promoter regions predicted by the sliding-window approach and PATSER (satisfied 80 % and 7.0 of sliding window identity bound and PATSER weight score)

Effector	MicroRNA	Binding site	% Identity (sliding window)	Score (PATSER)
P22260	ath-MIR408	TTGATGTGGGTCAA	92.98	12.08
		TTGACATTATTCAA	90.90	8.48
		AGTGTGTTTTGGATCACGGT	82.83	8.28
		TTGAATCCCATCCA	84.85	7.72
	ath-MIR168a	TTTGTGATTTTAATCCCACT	88.66	8.21
	ath-MIR169k	TATATGATAAAAATGTAACATA	86.79	8.74
	ath-MIR169l	TATATGATAAAAATGTAACATA	86.79	8.74
	ath-MIR771	TTGACGAGCCTCAA	87.87	7.51
Q8P8F9	ath-MIR164c	AGTAAACTGTTTCA	86.92	8.22
	ath-MIR169j	TGGAAACGCTTACA	92.30	9.17
	ath-MIR169k	AGAAAACGTATGCA	89.23	9.86
	ath-MIR169l	AGAAAACGTATGCA	89.23	9.86
	ath-MIR408	TTAAAAAGTTTTCA	87.69	8.56

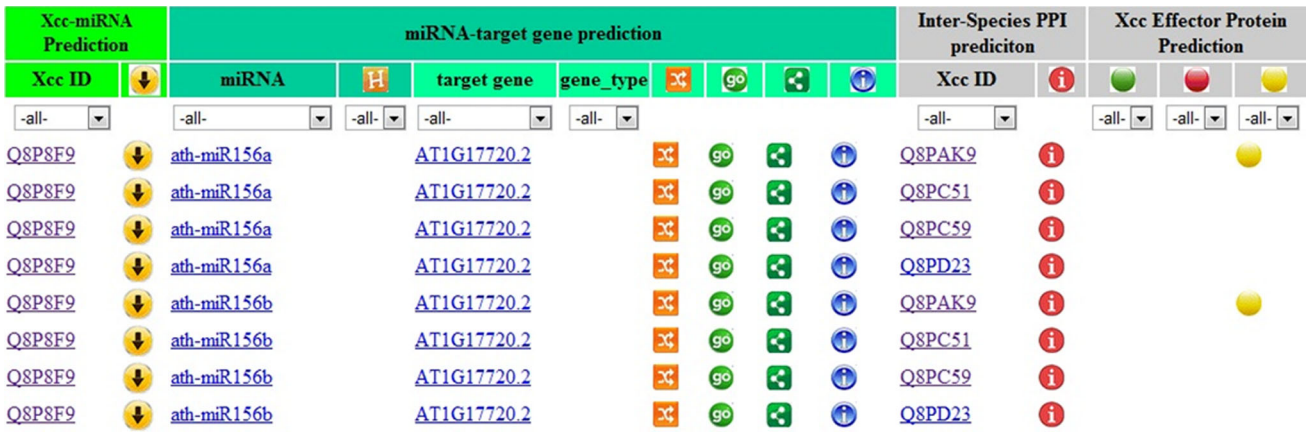

Fig. 4 Output screen of the web service

microRNA is disturbed, its effect may be amplified downstream, degrading the host antibacterial defense capability. For point (3), it is hypothesized that some of the pathogen proteins may strongly interact with the host PRG or TF, resulting in suppression of the host immune system.

Given the *Xcc* effector protein ID, microRNA ID, or target gene, the web site will return useful outputs, such as: (1) the putative *A. thaliana* TFBS of *Xcc* effector; (2) the microRNA-regulated PPI pathways; and (3) the PPI networks for *Xcc* and *Arabidopsis*. The query page is shown in Fig. 4.

The database is freely accessible at: http://ppi.bioinfo.asia.edu.tw/EDMRP.

Conclusions

In this study, we integrated three sets of predicted results: (1) putative microRNA TFBS for *Xcc* effectors; (2) microRNA-regulated PPI pathways, and (3) PPI networks for *A. thaliana* and *Xcc*.

The three datasets were obtained by (1) adopting interolog and TFBS profile information; (2) implementing machine-learning algorithms; and (3) computing protein interaction between *A. thaliana* and *Xcc* bacteria using the interolog and the domain–domain interaction methods. These three datasets were integrated as shown in Fig. 2; upstream are the effector–DNA interaction and the microRNA-regulated protein interaction of *A. thaliana* pathways, which are focused on PRG and TF; downstream is the PPI of *Xcc* and *A. thaliana*.

Pathogen–host interactions remain a largely unexplored area in computational biology. The present work is probably the first one in constructing pathways that comprise effector, microRNA, target genes and PPI for the study of pathogen–host interactions. This current study may provide key information to help reveal the biological processes involved in the immune systems of plants when under attack by bacteria.

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Conflict of interest No conflict of interest.

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