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Gene expression analysis of a chitin-induced receptor like kinase using expression database

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Abstract: Receptor-like kinases family were involved in various biological process in plant. Chitin-induced receptor like kinase 1 (CRLK1) was previously re-ported to play a role in resistance to fungal pathogens. In this study, CRLK1 was shown to be induced by various elicitors derived from bacteria, fungi and plant cell wall fragments. CRLK1 was also induced by several pathogens including bacteria, fungi, oomycetes and insect. These findings indicate CRLK1 may function as an intermediate regulator in plant defense pathway.

Key words: Receptor-like kinase; Fungal pathogens; Bacterial pathogens; Co-expressed gene network.

Introduction

Receptor-like kinases (RLKs) are a family of genes that involved in various plant biological processes, such as development, innate immunity, cell differentiation and patterning. Many RLKs have been reported to be cru-cial in plant defense pathways induced by pathogen-as-sociated molecular patterns (PAMP) (1). The most well-known RLKs that recognize elicitors are FLS2, CERK1/ LysM RLK1, EFR, WAK1 and the corresponding elicitors for these RLKs are flg22, chitin, ef-tu and Oligogalacturo-nide(OG)(2-6). In addition to the primary RLKs directly interacting with elicitors, numerous RLKs are present in plants and play a critical role in interacting with primary RLKs and activating downstream signaling pathway(1). Some RLKs are also up-regulated by elicitors via prima-ry RLKs such as FLS2, CERK1//LysM RLK1, EFR and WAK1, though their functions are still unknown.

Chitin-induced receptor like kinases 1 (CRLK1, TAIR accession No. At5g46080) was reported as a RLK up-re-gulated by chitin oligomers(5). Mutation in CRLK1 genes resulted in susceptibility to both biotrophic and necrotro-phic fungal pathogens(7). So far, the expression patterns of CRLK1 to other elicitors and pathogens have not been investigated. In this study, the expression patterns of CRLK1 was investigated using public available microar-ray and RNAseq data. The potential function involved in CRLK1 was also discussed in the context of plant defense pathways.

Materials and Methods

Identify induced gene expression level of CRLK1

Genevestigator was used to identified the gene expres-sion levels under biotic and elicitors conditions (8). Ge-nevestigator is a very powerful tool based on public avai-lable microarray and RNAseq data and can provide useful information for gene expression patterns. We download the program from Genevestigator website (https://gene-vestigator.com/gv/) and used academic license for diffe-rential expression tool. The accession number At5g46080 was submitted in the software to request the expression data from various pathogens and elicitors. The values of gene expression were manually recorded and saved in a local computer.

Co-expressed gene network of CRLK1

Co-expressed network for CRLK1 was obtained from Arabidopsis thaliana trans-factor and cis-element predic-tion database (ATTED-II)(9). ATTED-II provide lists and networks of co-expressed genes from 58 publically avai-lable experiments. To obtain the co-expressed genes, AT-TED-II obtained used Pearson's correlation coefficients to quantify the relationship between each pair of genes and the most correlated genes were used to generate the co-expressed genes network.

Gene ontology (GO) analysis of CRLK1 co-expressed genes

A total number of 20 CRLK1 co-expressed genes were analyzed via GO Term Enrichment tool on Gene Ontolo-gy Consortium ((http://geneontology.org/). The terms that are significantly enriched were compared with the entire Arabidopsis reference genes with p-values less than 0.05.

Data analysis and statistical analysis

The means and standard deviation of log2 expres-sion values were obtained from detailed view of selected perturbations. The log 2 ratio of expression values were calculated by subtracting log2 value of expression expe-riments from that of control groups. Student t tests were used to test the differences of expression levels among different treatments. All tests were conducted using SAS 9.4 (SAS institute, Cary, NC).

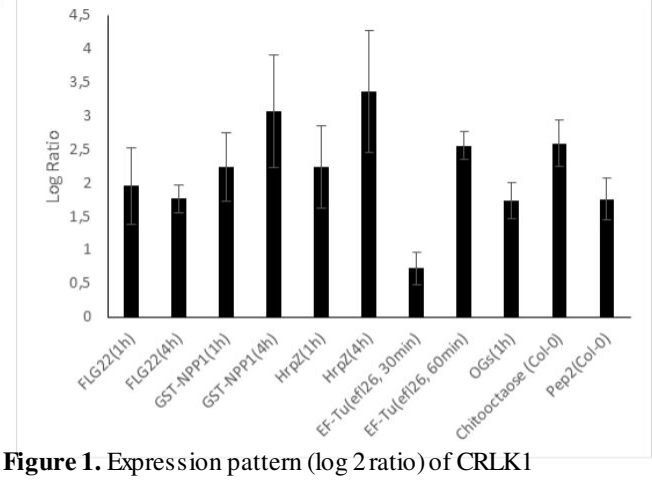

Figure 1. Expression pattern (log 2 ratio) of CRLK1 stimulated by various elicitors.

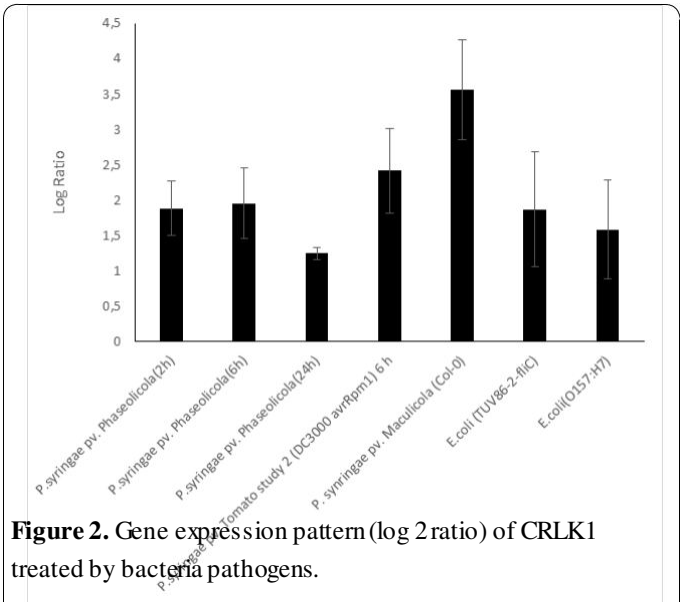

Figure 2. Gene expression pattern (log 2 ratio) of CRLK1 treated by bacteria pathogens.

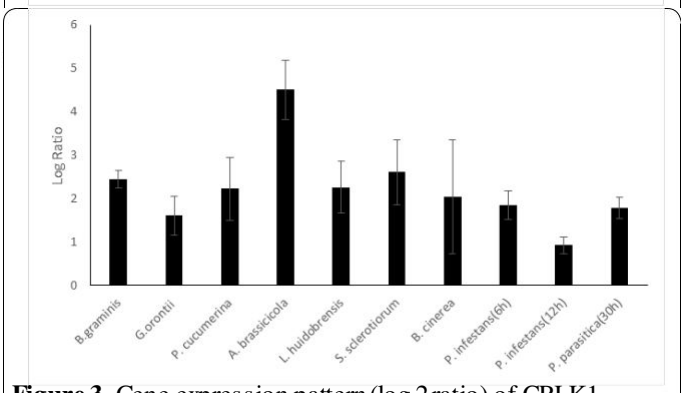

Figure 3. Gene expression pattern (log 2 ratio) of CRLK1 treated by fungal pathogens.

Results and Discussions

CRLK1 was first identified from RLK family gene list that are induced by chitin. It has been shown that chitin failed to induce CRLK1 expression in the CERK1 mu-tant(5), indicating that CRLK1 may work downstream the Arabidopsis defense pathway. However, chitin is not the only elicitor that induces CRLK1. As figure 1 shows, CRLK1 was also induced significantly by several other elicitors such as FLG22, GST-NPP1, HrpZ, EF-tu, OGs and Pep2, which indicate that CRLK1 may be involved in resistance to bacteria. Once Arabidopsis was treated with elicitors, CRLK1 responds quickly in 1 hour and the ex-pression level increased to more than 2 folds (log 2 ratio >1), and the expression continue to increase after 4 hours of treatment (Fig. 1).

When treating Arabidopsis with various bacteria pa-thogens, CRLK1 expression was also induced signifi-cantly. Interestingly, the level of CRLK1 responding to P. syringae pv. Phaseolicola started to decline at 24 hours of treatment. Similarly, the expression of CRLK1 was induced at a high level at the beginning of chitin treat-ment, and then gradually decreased to the normal level after 4 hours(7). Therefore, CRLK1 may act as an inter-mediate module to passing defense signals to downstream pathway, and CRLK1 expression were tuned back to nor-mal once CRLK1 activate other molecular partners. As ex-pected, CRLK1 was induced significantly by a variety of fungal pathogens (B. graminis, G. orontii, P. cucumerina, A. brassicicola, S. sclerotiorum, B. cinerea), oomycetes (P. infestans and P. parasitica) and insect (L. huidobrensis) (Fig. 3).

To identify genes represented on the microarray chip that show significant co-expression with CRLK1, an ex-pression correlation analysis with the CoExSearch tool implemented in ATTED-II was conducted(9). Three genes are directly co-expressed with CRLK1: Leucine-rich repeat protein kinase family protein (At1g74360), cysteine-rich RLK (RECEPTOR-like protein kinase) 11 (At4g23190), UDP-glucosyltransferase 71B6 (At3g21780). Other genes co-expressed with CRLK1 include receptor kinases, hy-drolase, and phytosulfokine receptor (Fig. 4).

The GO analysis showed the CRLK1 co-expressed ge-nes were enriched significantly for protein phosphoryla-tion, cellular protein modification and metabolic process when searching in biological process (Table 1). The co-ex-pressed genes were significantly enriched in carbohydrate

Table 1. GO analysis of the co-expressed genes of CRLK1.

Term	Background frequency	Sample frequency	p-value
Biological Process			
protein phosphorylation (GO:0006468)	685	12	1.37E-11
cellular protein modification process (GO:0006464)	1577	13	8.82E-09
cellular protein metabolic process (GO:0044267)	2204	13	5.84E-07
Molecular function			
carbohydrate binding (GO:0030246)	252	6	3.32E-05
protein serine/threonine kinase activity (GO:0004674)	584	12	1.51E-12
ATP binding (GO:0005524)	1701	15	1.44E-11
Cellular component			
plasma membrane (GO:0005886)	3033	14	3.97E-07
integral component of membrane (GO:0016021)	4044	15	1.20E-06

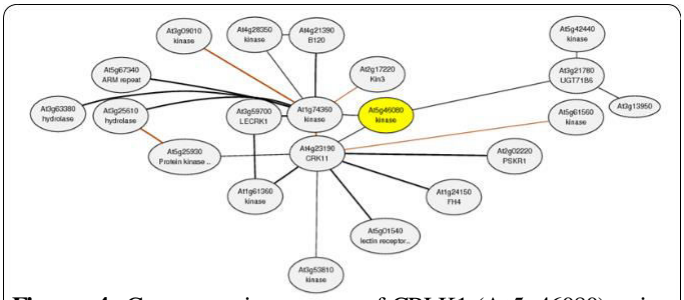

Figure 4. Co-expression genes of CRLK1 (At5g46080) using CoEx-Search tool implemented in ATTED-II.

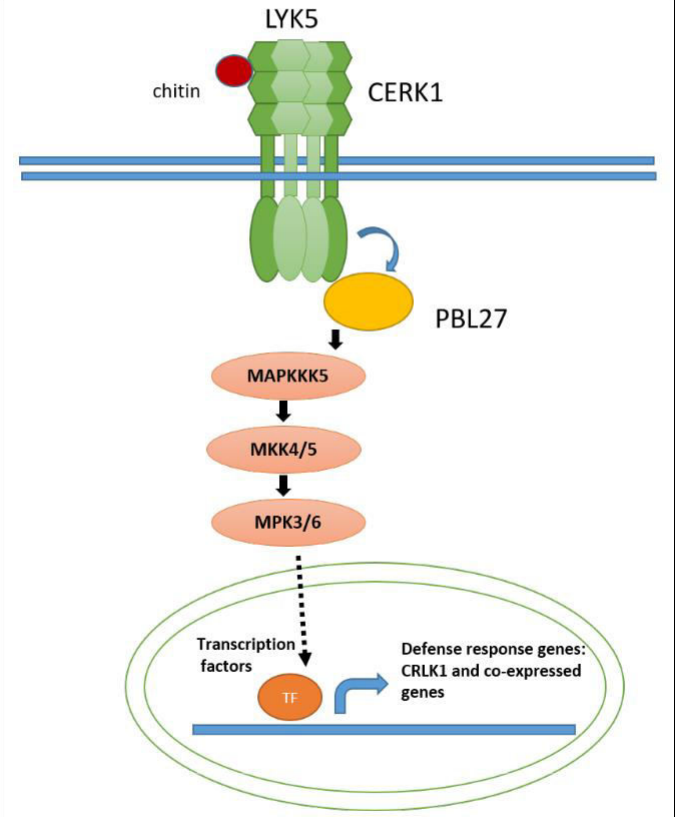

Figure 5. A possible working model for CRLK1 in chitin-induced plant defense pathway. Upon recognition of chitin, CERK1 and LYK5 activate PBL27 which then phosphorylate MAPKKK5. MAPKKK5 activate downstream MKK and MPK signaling pathway and induce CRLK1 and co-expressed genes through transcription factors.

binding, protein serine/threonine kinase activity and ATP binding functions. The co-expressed genes were signifi-cantly enriched in plasma membrane and integral com-ponent of membrane. These results indicate that CRLK1 mainly co-expressed with protein kinases that might loca-lize to plasma membrane. Most of the co-expressed genes were not well studied, and it is highly possible that these genes are also involved in plant defense pathways against plant pathogens.

CRLK1 is an Arabidopsis receptor-like kinases in-duced by various elicitors and pathogens. The function of CRLK1 may be involved in mediate plant defense signa-ling pathway and turn on plant defense mechanism.

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