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Co-regulatory genes of CEBiP: a stress responsive gene for rice blast infection

Abstract

CEBiP is the major binding receptor for chitin elicitors and provides resistance against *Magnaporthe oryzae*, the causal agent of rice blast disease. Chitin elicitors lead to various defense responses in plants. CEBiP has been shown to interact with chitin elicitors and trigger the downstream defense responses. Here we identified genes that are co regulated with CEBiP and may play a role in defense mechanism for blast infection.

Microaray data were downloaded from NCBI GEO database and analyzed using R and bioconductor packages. K mean clustering was performed on the datasets and pearson correlation coefficients were calculated for genes falling in same cluster as CEBiP using SPSS. Genes identified for leaf tissues showed high correlation. For the dataset of blast infected root tissue, most of the identified genes in cluster were also correlated. The results suggest that defense mechanism to counteract chitin elicitor during blast infection is slightly different in leaf and roots, as the identified genes for both the tissues are different. Further, most of the identified genes have not been previously reported to be involved in rice defense and hence are novel targets for future studies.

Keywords: rice blast, microarray, co expression, stress response, chitin induced response

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Introduction

Plants activate defense systems upon the recognition of microbe-associated molecular patterns (MAMPs). These are small, structurally conserved molecules within microbial species. Pattern recognition receptors (PRRs) located on the plasma membrane of plant cells, recognise the MAMPs.[1–4] The PRRs are the receptor-like protein kinases (RLK) and receptor-like proteins (RLP) localized in the plasma membrane.[3] Chitin is a constituent of fungal cell walls and acts as a carbohydrate MAMP, and elicits various defense responses in many plant species.[5]

In rice, CEBiP and OsCERK1 play key roles in the perception of chitin oligosaccharides.[6,7] LYP4 and LYP6 are also known to play role in the recognition of chitin in rice.[8] LYP4 and LYP6 are also reported to bind peptidoglycan, and function as the receptors for both chitin and peptidoglycan. Knockdown of CEBiP, LYP4 or LYP6 expression have been shown to cause increase in the spread of the infection hyphae of the blast fungus.[4,8] In the present study, we identified additional genes that may be involved along with CeBIP in defence response using microarray data.

Materials and methodology

Dataset collection

The microarray gene expression data from gene expression ominous (GEO) database at NCBI was downloaded. For comparison, we have choosen data from leaf and root tissues.

Data analysis

Microarray data downloaded were analysed with bioconductor packages using R. The method used for affymetrix data (CEL files) normalization was Robust multi-array analysis (RMA)[9] in the affy Bioconductor package. Functions for normalizing two-color agilent data are available in package limma; lowess (or loess) function was used. Further, k mean clustering was performed on whole datasets. Clusters having CeBIP in both the dataset were indentified and pearson correlation coefficient were calculated for genes of the same cluster using SPSS. The genes having correlation value >0.75 were selected as coexpressed.

Further analysis was done using blast2GO tool. mRNA Sequences downloaded from genbank were used as input and the coregulated genes were identified for the associated gene ontology terms. Also enzyme coding and kegg mapping options of the tool allowed to identify the already known pathways if any associated with the co-regulated genes.

Results

Identification of co-regulated genes

It is assumed that the genes regulated similarly would have correlating expression patterns. We attempted to identify target genes for rice blast using microarray datasets. To investigate the correlations between genes in the datasets, pearson correlation analysis was used that gives the measure for how well the two genes correlate. The correlation tables for leaf and root data are shown in Table 1 & 2.

Three hypothetical genes (Os01g0566100, Os08g0242700 and Os10g0509100) were co regulated with CEBiP, along with Os05g0119300 (Glycine-rich protein A3), Os03g0222100 (MA3 domain-containing protein, putative, expressed) and Os07g0580500 (BZR1) in leaf tissue. The gene descriptions were downloaded from Oryzaexpress database (http://plantomics.mind.meiji.ac.jp/OryzaExpress/).

Table 1 Correlation coefficients for genes falling in same cluster as Cebip (Os03g0133400) in leaf tissue

The genes with correlation values higher than 0.75 are shown in bold

7:OS 07G 0580500	0.977	0.965	0.964	0.979	0.976	0.961	1
6:OS10G 0509100	0.985	0.969	0.99	0.991	0.963	1	0.961
5:OS08G 0242700	0.985	0.988	0.976	0.973	1	0.963	0.976
4:OS03G 0 222100	0.989	0.97	0.991	1	0.973	0.991	0.979
3:OS03G 0133400	0.989	0.985	1	0.991	0.976	0.99	0.964
2:OS05G 119300	0.986	1	0.985	0.97	0.988	0.969	0.965
1:OS01G 0566100	1	0.986	0.989	0.989	0.985	0.985	0.977
	1:OS01G 0566100	2:OS05G 0119300	3:OS03G 0133400	4:OS03G 0222100	5:OS08G 0242700	6:OS10G 0509100	7:OS07G 0580500

Following datasets were retrieved from GEO for analysis.

S. no.	GSE ID	Description
1	GSE8518	Rice leaf sheath at 36 hrs after inoculation rice with blast fungus *M. oryzae* using agilent array technologies.[12]
2	GSE18361	Infected (with *M. oryzae*) and non infected roots of rice at 2, 4, and 6 days post-inoculation using affymetrix technology.[13]

Table 2 Correlation coefficients for genes falling in same cluster as Cebip (Os03g0133400) in root tissue. The genes with correlation values higher than 0.75 are shown in bold

14:Os04g0674000	0.753	0.947	0.873	0.885	0.775	0.933	0.812	0.923	0.917	0.95	0.897	0.831	0.923	1
13:Os07g0557100	0.837	0.872	0.874	0.894	0.716	0.882	0.782	0.9	0.951	0.946	0.862	0.829	1	0.923
12:Os03g0757900	0.865	0.855	0.796	0.83	0.849	0.913	0.843	0.926	0.908	0.769	0.966	1	0.829	0.831
11:Os07g0581000	0.82	0.89	0.81	0.82	0.8	0.95	0.87	0.9	0.92	0.85	1	0.97	0.86	0.9
10:Os01g0292200	0.72	0.83	0.88	0.85	0.61	0.84	0.7	0.86	0.88	1	0.85	0.77	0.95	0.95
9:Os01g 0830700	0.86	0.93		0.93	0.87	0.95	0.91	0.95	1	0.88	0.92	0.91	0.95	0.92
8:Os01g 0290700	0.83	0.92	0.81	0.93	0.87	0.95	0.91	0.95	1	0.88	0.92	0.91	0.95	0.92
7:Os06g066	0.75	0.89	0.63	0.8	0.91	0.92	1	0.85	0.91	0.7	0.87	0.84	0.78	0.81
6:Os02g 072	0.8	0.96	0.77	0.86	0.88	1	0.92	0.92	0.95	0.84	0.95	0.91	0.88	0.93
5:Os03g 0133400	0.82	0.9	0.67	0.8	1	0.88	0.91	0.88	0.87	0.61	0.8	0.85	0.72	0.78
4:Os03g072	0.83	0.9	0.81	1	0.8	0.86	0.8	0.93	0.93	0.85	0.82	0.83	0.89	0.89
3:Os02g07 66700	0.871	0.81	1	0.811	0.669	0.767	0.628	0.847	0.812	0.875	0.81	0.796	0.874	0.873
2:Os07g01 76200	0.817	1	0.81	0.896	0.9	0.964	0.893	0.916	0.929	0.828	0.885	0.855	0.872	0.947
1:Os04g0244800	1	0.817	0.871	0.826	0.82	0.795	0.748	0.831	0.861	0.719	0.815	0.865	0.837	0.753
	1:Os04g0 244800	2:Os07g 0176200	3:Os02g 0766700	4:Os03g 072	5:Os03g 0133400	6:Os02g 072	7:Os06g66	8:Os01g0 290700	9:Os01g0 830700	10:Os01g 0292200	11:Os07g 0581000	12:Os03g 0757900	13:Os07g 0557100	14:Os04g 0674000

The co-expressed genes in root tissue were Os04g0244800 (heavy metal transport/detoxification protein domain containing protein), Os07g0176200 (tesmin/TSO1-like, CXC domain containing protein, Os03g0726500 (protein of unknown function DUF81 domain containing protein), Os06g0661900 (protein of unknown function DUF266), Os01g0290700 (similar to CjMDR1), Os01g0830700

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(protein of unknown function DUF231), Os07g0581000 (protein of unknown function DUF250), Os03g0757900 (similar to UDP-glucose 6-dehydrogenase), Os04g0674000 (similar to H0403D02.10 protein).

Gene Ontology analysis and pathway analysis

Blast2GO was used for analysis and the data are compiled in Table 3 & 4 for leaf and root tissue respectively. The results for BLAST analysis are against the rice protein database and then the GO (gene ontology) terms database. For leaf tissue data no enzyme codes were identified for KEGG pathways. Thus none of these genes can be predicted to be involved in any of the known KEGG pathways. CeBiP too did not have an enzyme code associated with it.

Table 3 Blast2GO results showing values for BLAST similarity search, GO terms, enzyme code analysis and KEGG pathway analysis for leaf tissue data

Description	Seq. length	#Hits	Min. eValue	Mean similarity	#GOs	GOs	Enzyme codes	KEGG pathway
Os12g0152700	1779	1	1.31E-35	72.00%	2	P, metabolic process; C, chloroplast part	-	-
Os10g0509100	1121	1	1.18E-113	100.00%	0	-	-	-
Os06g0552300	1206	3	1.29E-34	54.33%	4	P, carbohydrate metabolic process; F, catalytic activity; P, polysaccharide catabolic process; F, beta-amylase activity	-	-
Os08g0242700	926	0			0	-	-	-
Os03g0222100	2587	5	0	67.00%	1	P:RNA metabolic process	-	-
Os03g0133400	1511	7	0	55.43%	7	P, defense response; P, cell wall macromolecule catabolic process; C, integral component of membrane; F, chitin binding; F, protein self-association; C, cytoplasmic, membrane-bounded vesicle; C, plasma membrane	-	-
Os01g0566100	1681	1	0	100.00%	0	-	-	-

P represents associated biological processes, F represent molecular processes and C represent cellular components in GO description.

Table 4 Blast2GO results showing values for BLAST similarity search, GO terms, enzyme code analysis and KEGG pathway analysis for root tissue data.

Seq. description	Seq. length	#Hits	Min. eValue	Mean similarity	#GOs	GOs	Enzyme codes	KEGG pathway
Os07g0581000	1710	16	0	58.75%	4	P, response to nematode; C, intracellular membrane-bounded organelle; C, cytoplasmic part; C, integral to membrane	-	
Os07g0557100	1681	3	0	86.33%	3	P, transport; C, integral to membrane; F, nucleoside transmembrane transporter activity	-	
Os07g0176200	2760	6	0	64.00%	1	C, plastid		
Os06g0661900, partial	2021	14	0	62.50%	3	C, membrane; F, acetylglucosaminyltransferase activity; C, mitochondrion	EC:2.4.1	
Os04g0674000, partial	1145	1	5.01E-92	100.00%	1	C, plastid	-	
Os04g0244800	801	20	3.03E-110	65.90%	3	F, metal ion binding; P, metal ion transport; C, plasma membrane	-	
Os03g0757900	1942	3	0	98.67%	8	F, UDP-glucose 6-dehydrogenase activity; C, cytosol; F, NAD binding; C, cell wall; P, oxidation-reduction process; C, cytoplasmic membrane-bounded vesicle; P, UDP-glucuronate biosynthetic process; C, nucleus	EC:1.1.1.22	Amino sugar and nucleotide sugar metabolism Ascorbate and aldarate metabolism Pentose and glucuronate interconversions
Os03g0726500	2019	5	0	70.60%	3	C, cytoplasmic membrane-bounded vesicle; C, integral to membrane; C, mitochondrion	-	

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Table Continued....

Seq. description	Seq. length	#Hits	Min. eValue	Mean similarity	#GOs	GOs	Enzyme codes	KEGG pathway
Os03g0133400	1511	7	0	55.43%	7	P, defense response; P, cell wall macromolecule catabolic process; C, integral to membrane; F, chitin binding; F, protein self-association; C, cytoplasmic membrane-bounded vesicle; C, plasma membrane	-	
Os02g0766700	1827	9	4.02E-151	62.33%	7	F, transcription regulatory region DNA binding; P, response to abiotic stimulus; P, regulation of transcription, DNA-dependent; F, sequence-specific DNA binding; F, sequence-specific DNA binding transcription factor activity; P, abscisic acid mediated signaling pathway; P, response to stress	-	
Os02g0721700	1012	5	9.49E-91	72.80%	6	P, pollen tube reception; P, pollen tube guidance; C, cytoplasmic membrane-bounded vesicle; C, plasma membrane; P, synergid death; P, double fertilization forming a zygote and endosperm	-	
Os01g0830700	2019	20	0	63.20%	6	C, mitochondrion; P, cellular macromolecule metabolic process; C, plastid; P, cell wall organization or biogenesis; P, response to freezing; P, polysaccharide metabolic process	-	
Os01g0292200	1785	20	0	67.25%	11	P, response to salt stress; F, protein tyrosine kinase activity; P, protein phosphorylation; F, protein serine/threonine kinase activity; P, signal transduction; P, response to inorganic substance; P, response to extracellular stimulus; P, response to abscisic acid stimulus; C, plasma membrane; F, ATP binding; C, mitochondrion	EC:2.7.10; EC:2.7.11	
Os01g0290700, partial	730	20	4.62E-93	77.95%	23	P, root hair elongation; C, vacuolar membrane; P, ATP catabolic process; P, response to cytokinin stimulus; C, chloroplast envelope; P, response to aluminum ion; P, auxin efflux; C, cytoplasmic membrane-bounded vesicle; C, plasma membrane; P, gravitropism; C, integral to membrane; F, auxin efflux transmembrane transporter activity; C, mitochondrion; C, ATP-binding cassette (ABC) transporter complex; P, acropetal auxin transport; F, ATP binding; P, basipetal auxin transport; F, xenobiotic-transporting ATPase activity; P, response to stress; C, plant-type vacuole; P, cellular metal ion homeostasis; P, transmembrane transport; F, oligopeptide-transporting ATPase activity	EC:3.6.3.44; EC:3.6.3.23	

P represents associated biological processes, F represent molecular processes and C represent cellular components in GO description.

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For root tissues few of the coregulated genes were identified with enzyme codes and also Kegg Pathways associated with one of them have been provided by Blast2GO tool. Os03g0757900 (similar to UDP-glucose 6-dehydrogenase) was identified with KEGG pathways; amino sugar and nucleotide sugar metabolism, ascorbate and aldarate metabolism, pentose and glucuronate interconversions. Thus we can predict that the identified coregulated genes must also share common functions associated with these pathways.

Discussion

Chitin is a well-known inducer of immune responses in plants and significant advances in knowledge of the molecular mechanisms of chitin perception and chitin-triggered immunity in plants have been achieved.[10] However, these processes are still not completely known. The different genes involved along with the signaling components remain largely unknown.

CeBIP, OsCERK1, OsRAC1, OsLYP4 and OsLYP6, have been previously reported to be involved in the defence mechanism but this knowledge is limited.[11] Using coexpression analysis we established a direct link between the genes that must be coregulated and can be further analysed and verified using wet lab techniques. According to our analysis a defined pathway for chitin induced defence response is still not available and hence need extensive work to be done in this regard. Also all the genes identified in our study provides a strong starting point for the same.

Conclusion

Microarray analysis is a high throughput technique that leads to the generation of a large amount of data and can be used by other researchers for further analysis. Here we downloaded freely available data from NCBI GEO database and identified coexpressed genes of CeBIP in rice and root tissues. The genes identified were different for the two datasets. Our study provides an insight into the chitin induced defence mechanism. Further the genes identified are novel targtes for further studies as they have not been identified in chitin induced defence mechanism previously.

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Conflict of interest

The author declares no conflict of interest.

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