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.

Microarray 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

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, recognize 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 defense response using microarray data.

Materials and Methodology
Dataset collection
The microarray gene expression data from gene expression omnibus (GEO) database at NCBI was downloaded. For comparison, we have chosen 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 identified 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.

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.
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.

| S.No. | GSE ID     | Description                                                                 |
|-------|------------|------------------------------------------------------------------------------|
| 1     | GSE8518    | Rice leaf sheath at 36 hrs after inoculation rice with blast fungus.          |
| 2     | GSE18361   | Infected (with M. oryzae) and non infected roots of rice at 2, 4, and 6 days |
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.

| Gene       | Corr Value | Description |
|------------|------------|-------------|
| 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 |
| 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 |
| 12:Os03g0759000 | 0.865 | 0.855 0.796 0.83 0.849 0.913 0.843 0.926 0.908 0.769 0.966 1 |
| 11:Os07g0581000 | 0.82 | 0.89 0.81 0.82 0.8 0.95 0.87 0.9 0.92 0.85 1 |
| 10:Os01g0292200 | 0.72 | 0.83 0.88 0.85 0.6 0.84 0.7 0.86 0.88 1 0.85 0.77 0.95 0.95 |
| 9:Os04g0830700 | 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:Os04g0290700 | 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:Os02g072 | 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:Os02g0133400 | 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:Os02g0769000 | 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:Os07g0176200 | 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:Os04g0244800 | 2:Os07g0176200 | 3:Os02g0769000 | 4:Os03g072 | 5:Os03g0133400 | 6:Os02g072 | 7:Os06g066 | 8:Os01g0290700 | 9:Os01g0830700 | 10:Os01g0292200 | 11:Os07g0581000 | 12:Os03g0759000 | 13:Os07g0557100 | 14:Os04g0674000 |

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; E:carbohydrate metabolism; P:polysaccharide catabolic process; F:beta-amylase activity | - | - |
| Os08g0242700 | 926 | 0 | 0 | 0 | 67.00% | 1 | P:RNA metabolic process | - | - |
| Os03g0222100 | 2587 | 5 | 0 | 67.00% | 1 | P:RNA metabolic process | - | - |
| Os03g0133400 | 1511 | 7 | 0 | 55.43% | 7 | P:cellular metabolic process; C:cell wall macromolecule catabolic process; F:cell wall macromolecule catabolic process; C:cellular component of membrane; F:cell wall macromolecule catabolic process; F:cell wall macromolecule catabolic process | - | - |
| 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; Facetylglicosaminyltransferase activity; C:mitochondrion | EC:2.4.1.1    |
| Os04g0874000, 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:nitration-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 |
| Os03g0765500     | 2019        | 5     | 0           | 70.60%          | 3    | C:cytoplasmic membrane-bounded vesicle; C:integral to membrane; C:mitochondrion | -             |              |
| Os03g0133400     | 1511        | 7     | 0           | 55.43%          | 7    |                                                                      | -             |              |
| 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; F: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:syncellular 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 | -             |              |

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### 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 targets for further studies as they have not been identified in chitin induced defence mechanism previously.

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