

# In silico analysis and molecular characterization of rice oxalate oxidase, a candidate gene associated with quantitative resistance to rice blast

Converging evidence points to a role for oxalate oxidases in the defense response of plants to fungal pathogens. Oxalate oxidase is an  $H_2O_2$ -generating enzyme catalyzing the reaction:  $oxalate + O_2 + 2H^+ \rightarrow 2CO_2 + H_2O_2$ . Active oxygen species, such as  $H_2O_2$ , have been suggested to be involved in plant defense responses against several fungal pathogens. Oxalate oxidase is a member of the cupin superfamily of functionally diverse proteins with the conserved domains G(x)5HxH(x)3,4E(x)6G and G(x)5PxG(x)2H(x)3N (PFAM00190). Using the candidate gene approach, oxalate oxidase and oxalate oxidase-like proteins have been linked to resistance to rice blast (*Magnaporthe grisea*). With the complete sequence of the rice genome, along with the genetic and bioinformatic resources for rice, our ability to comprehensively characterize gene families has also been greatly enhanced. These resources include the physical, linkage, and expressed sequence tag (EST) maps, a collection of >130,000 ESTs, gene prediction algorithms, and online resources for comparative grass genomics. Through these resources, we characterized by *in silico* analysis oxalate oxidase in rice, a candidate gene conferring quantitative resistance to rice blast.

## IDENTIFYING OXALATE OXIDASE SEQUENCES IN THE RICE GENOME

We scanned the rice genome (<http://www.tigr.org/tdb/e2k1/osa1>) for oxalate oxidase and oxalate oxidase-like proteins by BLAST using the barley clones Y14206 (oxalate oxidase) and X93171 (oxalate oxidase-like protein). To retrieve similar sequences from the genome database, we also used a perl script that would identify sequences with the cupin conserved domain. We identified 74 rice proteins with the cupin motifs. Phylogenetic analyses group these proteins into two major clusters: the germin-like proteins (GLP) and cupins with other functions. We focused on the GLPs, where oxalate oxidase and other germins associated with resistance to fungal pathogens group.

## PHYLOGENETIC ANALYSIS OF RICE CUPIN SEQUENCES

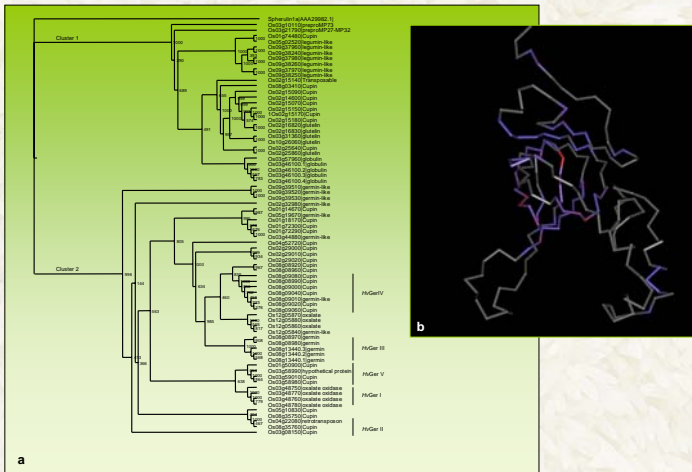


Fig. 1. (a) Phylogenetic relationships of the cupin superfamily of proteins in the genome of rice cultivar Nipponbare. Oxalate oxidase is a member of the cupin superfamily of proteins with PFAM00190 domain. There are 74 cupin sequences in the rice genome. Two main clusters are derived based on protein sequences. Cluster 1 is composed of large cupins such as glutelins and globulins. Cluster 2 is composed of germin-like proteins such as oxalate oxidase. Two types of germin have been linked to blast resistance in rice, Chr3 oxalate oxidase and Chr8 oxalate oxidase-like protein, both of which occur in multicopies in the genome. These oxalate oxidase gene sequences are located next to each other, with similarities ranging from 90 to 98%. For each gene, there was variation in the copy number of *cis*-elements related to biotic stress responses, such as W box, WNPR1, and WRKY, indicating that these genes have potential associations with the response of rice to pathogen infection, such as the blast fungus. The tree was generated by distance matrix using ClustalX and rooted with spherulinf1a. Groupings matching the classification of Druka et al (2002) for germin-like proteins in barley are indicated on the right. (b) Three-dimensional structure of the cupin domain (<http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=pfam00190>).

## DISTRIBUTION OF CUPIN SEQUENCES IN THE RICE GENOME



Fig 2. Chromosomal locations of cupin sequences in the genome of rice cultivar Nipponbare. The cupins are distributed across 9 chromosomes, as singletons or clusters of highly similar genes. A cluster of oxalate oxidases in chromosome 3 are associated with variation in resistance (i.e., lesion numbers) in a rice blast-resistant mapping population derived from Vandana/Moroberekan (Wu et al 2004) and are highly similar to a barley oxalate oxidase (gi2266667), previously reported to encode a cell wall-localized oxalate oxidase involved in the response to powdery mildew infection. A cluster of germin-like proteins in chromosome 8 has similarity with barley oxalate oxidase-like protein (gi1070357) and is also linked to quantitative resistance to rice blast in TXZ/SH22 mapping population (Liu et al 2004). The figure was generated by GenomePixelizer (Kozik et al., 2002). Lines connecting sequences indicate that these sequences have >75% identity.

## PROTEIN CHARACTERIZATION OF RICE CUPIN SEQUENCES

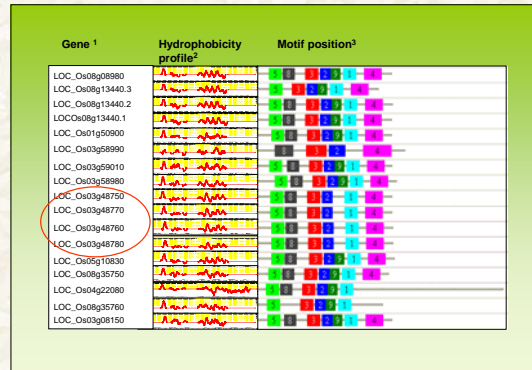


Fig. 3. Hydrophobicity profiles and motif positions of cupin sequences in the rice genome. We analyze each protein sequences for different parameters such as hydrophobicity, motif, molecular weight, and signal peptides to determine similarity with known cupins. Common to all germin-like sequences is motif 9 identified by MEME with the exception of the oxalate oxidases. The very high similarity of the four oxalate oxidases are also reflected in their similar hydrophobicity profiles. <sup>1</sup> Gene ID from Rice Genome Program; <sup>2</sup> Hydrophobicity was calculated using Kyte-Doolittle algorithm (scan window = 21); <sup>3</sup> Motif position as determined by MEME (Bailey and Gribskov, 1998) using protein sequences.

## SEQUENCE ANALYSIS OF CHROMOSOME 3 OXALATE OXIDASES IN DIVERSE RICE GERmplasm

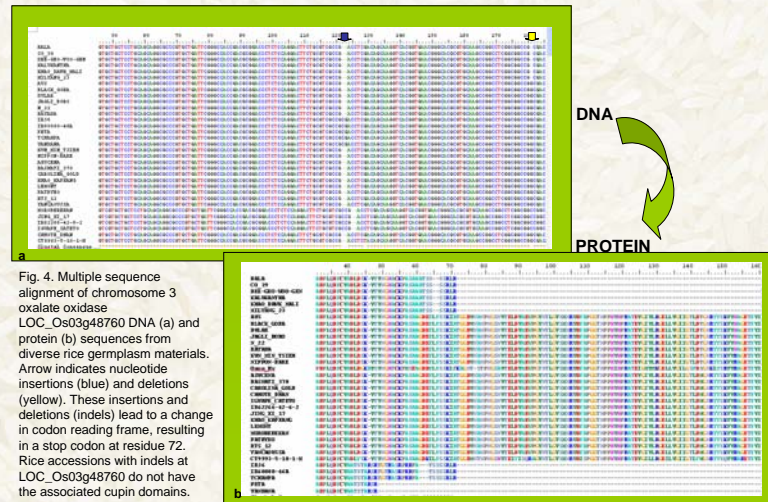


Fig. 4. Multiple sequence alignment of chromosome 3 oxalate oxidase LOC\_Os03g48760 DNA (a) and protein (b) sequences from diverse rice germplasm materials. Arrow indicates nucleotide insertions (blue) and deletions (yellow). These insertions and deletions (indels) lead to a change in codon reading frame, resulting in a stop codon at residue 72. Rice accessions with indels at LOC\_Os03g48760 do not have the associated cupin domains.

## CONCLUSIONS

- The cupin superfamily of proteins is well-represented with 74 members in the rice genome.
- There are two major cupin groups in the rice genome - the seed storage proteins and the germin-like proteins (GLP) - which are distributed as singletons or in clusters.
- A cluster of GLPs in chromosomes 3 (oxalate oxidases) and 8 (oxalate oxidase-like proteins) is associated with resistance to fungal pathogens.
- Sequence polymorphisms in oxalate oxidase LOC\_Os03g48760 lead to change in codon reading frame, resulting in truncated predicted proteins in several rice accessions.

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