



Phylogenomic analyses of rice oxalate oxidases, candidate genes for quantitative resistance to rice blast

Expression evidence from wheat, barley, sunflower, and peanut as well as genetic mapping studies suggest an important role for oxalate oxidases (OXO) in conferring resistance to fungal pathogens. OXOs are members of the cupin superfamily of proteins containing the two conserved motifs ([G(X)5HXH-(X)3,4E(X)6G] and G(X)5PXG(X)2H(X)3N]. Plant cupins are involved in developmental and defense response pathways. There are two subclasses of cupins in plants—the monocupin subclass which consists of a single cupin domain, and the bicupin, which has two copies of the cupin domain. OXOs are monocupins and are unique in cereals. They degrade oxalate to CO₂ and H₂O₂. Active oxygen species, such as H₂O₂, have been suggested to be involved in plant defense responses against several fungal pathogens. Using the candidate gene approach, oxalate oxidase and oxalate oxidase-like proteins have been linked to resistance to rice blast (*Magnaporthe oryzae*). In rice, OXO is mapped to chromosome 3 and is associated with resistance to rice blast in a Vandana/Moroberekan mapping population. With the availability of the complete genome sequence of rice, we undertook the task of characterizing rice monocupins including the four OXO genes present on chromosome 3 of the *O. sativa* 'Nipponbare' genome. The goal of this study was to confirm genetic mapping data from several populations for the presence of OXO genes in the rice genome, to characterize the members of the oxalate oxidase family, and to validate the role of these genes in response to pathogen infection.

Phylogeny of oxalate oxidases in cereal genomes

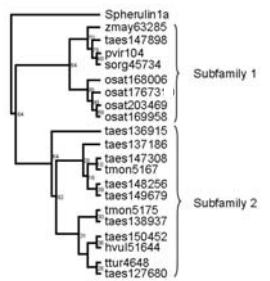


Fig 1. Phylogenetic relationships of cereal oxalate oxidases. The cereal OXO are unique in cereals. There are two OXO subfamilies based on amino acid sequences. Subfamily 1 consists of rice, wheat, switchgrass, sorghum, and maize sequences. All rice oxalate oxidases belong to subfamily 1 and sequences osat203469, osat169958, osat176731, and osat168006 correspond to LOC_03g48780, LOC_03g48770, LOC_03g48760, and LOC_03g48750, respectively. Subfamily 2 consists of wheat and barley oxalate oxidases. Amino acid sequences were derived from Phytome (<http://www.phytome.org>)

Phylogeny and genome organization of cupins in the rice genome

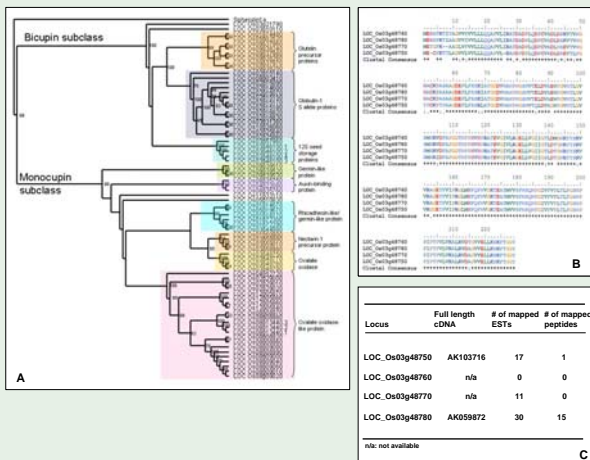


Fig. 2. Genome organization and phylogenetic analysis of OXO in rice. OXOs are members of the monocupin subclass of proteins in the rice genome. A) Analysis of the rice genome revealed 44 monocupins divided into six subfamilies with different functions. The biocupin subclass is composed of seed storage proteins. B) Multiple alignment of the four tandemly duplicated Ch3 OsOXO genes reveal >90% similarity at the amino acid level. C) EST and full-length clones reported for rice OsOXO genes.

Hydrophobicity profiles and motif positions of monocupins in the rice genome

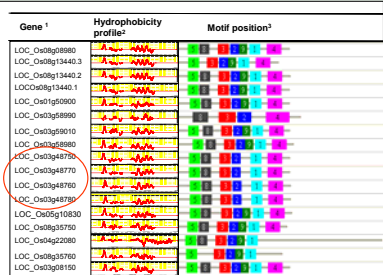


Fig. 3. Hydrophobicity profiles and motif positions of monocupin sequences in the rice genome. We analyzed each protein sequence for different parameters such as hydrophobicity, motif, molecular weight, and signal peptides to determine similarity with known cupins. Common to all monocupins is motif 9 with the exception of the oxalate oxidases. The high similarity of the four OXOs are also reflected in similar hydrophobicity profiles.

Expression analysis of oxalate oxidase genes in selected Vandana/Moroberekan advanced backcross lines

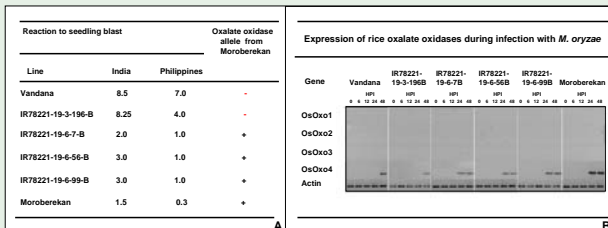
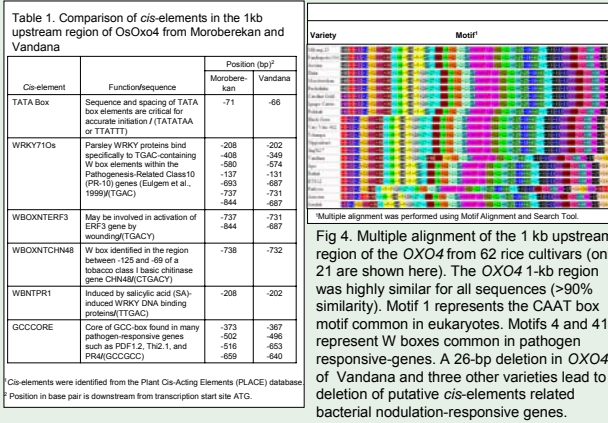


Fig. 3. Expression analysis by RT-PCR of OsOXO genes after inoculation of *Magnaporthe oryzae* isolate PO6-6 in selected advanced backcross lines of Vandana/Moroberekan. Lines were selected on the basis of their reaction to field blast in India and the Philippines, detection of the Moroberekan OsOXO allele in the progenies, and similarity of morphological and agronomic traits with Vandana, with the exception of IR78221-19-3-196-B which has the OsOXO allele from Vandana. Leaf samples were harvested at 0, 6, 12, 24, and 48 h post inoculation (HPI).

Analyses of the 1kb upstream region of OXO genes in rice



Summary and conclusion

- The cupin superfamily of proteins with 74 members is well-represented in the rice genome.
- There are two major cupin groups in the rice genome - the bicupins composed of seed storage proteins and monocupins which are composed of germins and germin-like proteins (GLP)
- OXOs are unique in cereals. There are four tandemly duplicated OXOs in chromosome 3 of rice. These genes are highly similar at both the nucleotide and amino acid levels.
- EST clones were identified only for OsOXO1, OsOXO3 and OsOXO4.
- Expression analysis using resistant and susceptible advanced backcross lines of Vandana x Moroberekan showed that only OsOXO4 is expressed during rice-Magnaporthe oryzae interaction
- Sequence analysis of the 1000-bp upstream region of each gene reveals variation in class and copy number and position of cis-elements involved in defense response.

References:

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 ZIMMERMANN, G., BAUMLEIN, H., MOCK, H.-P., HIMMELBACH, A., and SCHWEIZER, P. 2006. The Multigenic Family Encoding Germin-Like Proteins of Barley. Regulation and Function in Basal Host Resistance. *PLANT PHYSIOLOGY*, 142 1:181.

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