# Expression profiling and gene localization of rice lesion mimic mutant *spl1*

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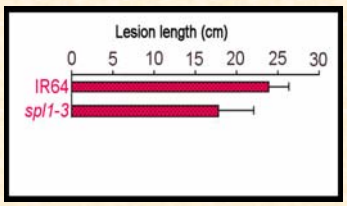
## Introduction

The rice lesion mimic mutant *spl1* shows enhanced resistance to important rice pathogens, i.e., *Magnaporthe grisea* (rice blast) and *Xanthomonas oryzae* pv. *oryzae* (bacterial blight). To study the broad spectrum resistance exhibited by this mutant, we examined changes in gene expression and initiated work to identify the gene responsible for the phenotype. Many lesion mimic mutants, which have been discovered in plant species including *Arabidopsis thaliana*, *Zea mays*, *Hordeum vulgare* and *Triticum sp*., show enhanced resistance to pathogen invasion.


Figure 1 (above) – Allelic series of lesion mimic mutant *spl1*. The orange/brown necrotic lesion of irregular shape are a hallmark of the mutation first identified in a spontaneous field mutant in 1970 by S. Kiwosawa (originally called Sekiguchi lesion or *sl* gene). Shown are the wild type (IR64) and deletion mutants generated by fast neutron (*spl1-2,* N-1856), gamma ray (*spl1-3,* GR-650), and diepoxybutane (*spl1-4,* D6-1137 & *spl1-5*, D6-2943). The leaves show variation in lesions at different points on the leaf and different lesion ages.

Figure 2 (below) – Lesion mimic mutant *spl1-3* shows enhanced resistance to the rice bacterial blight pathogen *X. oryzae* pv. *oryzae* as evidenced by the overall decrease in lesion length (cm). Plants were inoculated using the leaf-clipping method.




In a screen of more than 30,000 chemically and physically induced mutations in indica cultivar IR64, more than ten mutants with the *spl1* phenotype were found. The four mutants in Fig. 1 were shown to be the same gene by complementation. Inoculation with *X. o.* pv. *oryzae* showed reduced lesion length compared to IR64, the wild type (Fig. 2). For this reason we believe the lesion mimic mutants may provide valuable insight into rice defense responses.

## Expression profiling of *spl1-3*

Expression studies of *spl1-3* using Agilent's Rice Oligo Microarray (representing more than 22,000 full length cDNAs from the KOME full length cDNA database, http://cdna01.dna.affrc.go.jp/cDNA/) showed many genes differentially regulated across different leaf ages. To model changes in gene expression as a function of lesion development, the experiment included three sequential leaf positions from 45 day-old plants, with leaf one being the youngest fully expanded leaf. Differentially expressed genes included membrane associated kinases, defense response genes, genes responsible for maintenance of cell walls, sugar metabolism enzymes, transporters and photosynthetic genes. Though these analyses are still preliminary, interesting trends in gene expression were revealed.

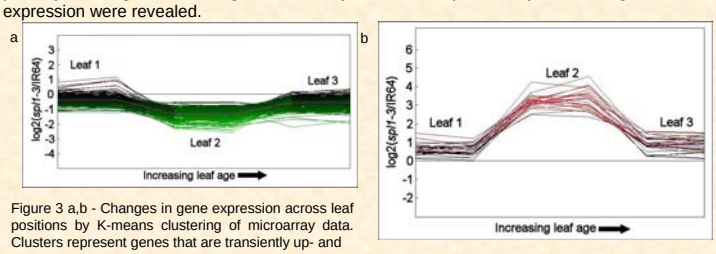



Figure 3 a,b - Changes in gene expression across leaf positions by K-means clustering of microarray data. Clusters represent genes that are transiently up- and down-regulated as lesion begin to form (leaf 2). Examples of down-regulated genes are an LRR membrane associated kinase, a calcium-dependent protein kinase, an ABC-type transporter and many genes of unknown function. Examples of up-regulated genes are PR1, cytochrome P450, sugar metabolizing enzymes, a myb transcription factor and many genes of unknown function.

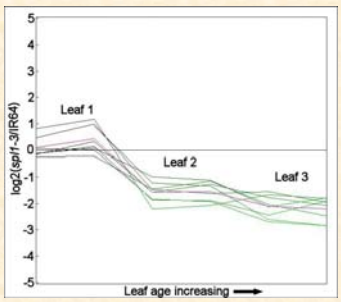



Figure 4 (above) – K-means clustering of genes showing down-regulation across leaf positions. These genes include a putative starch synthase, a serine/threonine wall-associated kinase, a serine protease, and an ankyrin repeat-like protein that may act as a transcription factor. In addition, a number of genes with unknown function were identified.

Many genes identified in the clusters are as yet of unknown function. However, as the picture of the rice transcriptome's response to the mutation develops, we may be able to assign putative functions or pathways to some these genes. Testable hypotheses of signaling as lesions develop based on these preliminary results could include cross-talk or differential regulation between competing signaling pathways.

Our preliminary analysis identified genes that may play important roles in aberrant lesion formation, and perhaps in the defense response. Confirmation of expression profiles of interesting genes is being done by using real time quantitative PCR (QT-PCR). For example, the defense response gene PR-1 is up regulated in leaf 2 of the lesion mimic mutant (Fig. 3b). This up regulation was confirmed by QT-PCR (Fig. 5).

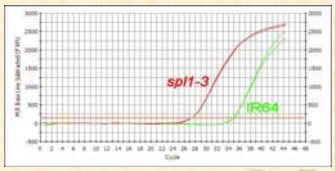



Figure 5 (right) – Real time quantitative PCR confirmation of PR1 up-regulation in lesion mimic mutant *spl1-3*.

## Gene localization

Recombination mapping studies placed *spl1* between 48.2 and 49.3 cM on chromosome 12. To discover the deleted gene(s), we hybridized sheared genomic DNA from four allelic lines with the rice *spl1* mutation to Syngenta's Rice GeneChip® Affymetrix array. A cluster of deletions was identified at 49.3 cM.

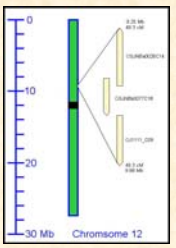



Figure 6 – Positions of BAC clones containing detected deletions in *spl1* allelic mutants on Chromosome 12.

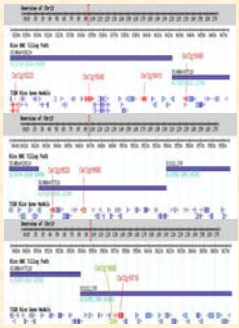


Table 1

| Syngenta probeset | TIGR model ID | Protein prediction (putative function) |
|---|---|---|
| Os1025420 | LOC_Os12g16130.1 | hAT family transposase dimerization domain |
| Os1013928 | LOC_Os12g16220.1, LOC_Os12g16220.2 | isoflavone reductase homolog/epimerase |
| Os1016625 | no model | isoflavone reductase-like (Syngenta model) |
| Os1050283 & Os1039203 | LOC_Os12g16340.1 | retrotransposon - unclassified |
| Os1022121 | LOC_Os12g16480.1 | galactosyl transferase |
| Os1027066 | LOC_Os12g16520.1 | wall-assoc. kinase |
| Os1043748 & Os1028480 | LOC_Os12g16690.1 | Zn finger C3HC4 type |
| Os1005492 | LOC_Os12g16710.1 | Ty1-copia class retrotransposon |

Figure 7– Probesets (a collection of oligonucleotide probes specific for gene prediction) detected as deleted in at least one *spl1* mutant (average IR64 signal/average mutant signal > 2 - **red**) and other gene models in the region (blue) mapped to the chromosome 12 BACs (**purple**). Table 1 correlates the Syngenta probeset names to TIGR v3 annotation gene models. Model in **green** did not meet criteria for a deletion call, but was later shown to be deleted by single probe analysis and PCR (see below).

The initial analysis to detect deleted genes (reduced hybridization of the rice genomic DNA to the array) was based on average intensity values for the probes in a probeset. This analysis allowed identification of the regions hybridizing to the probesets shown in **red** in Fig. 7 above as deleted, and confirmed these by PCR.

This analysis, however, did not detect some regions known to be deleted. The failure to detect deletions likely occurred because, in situations where one or a few probes from a probeset are deleted, the average of intensities for all probes in that set would not meet the criteria for a deletion call (IR64/mutant signal > 2). To circumvent this problem, we analyzed individual probe hybridization patterns to describe deletions.

**Fig. 8**

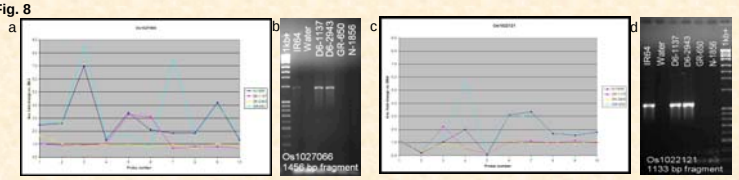



Figure 8 a,c – Individual probe intensity fold change calculated as average IR64/average mutant for probesets detected as deleted by probe intensity averaging. Values >1 indicate a reduction in mutant signal intensity. b,d) PCR confirmation of detected deletion in *spl1-3* (GR-650) and *spl1-2* (N-1856).

Figure 9–(right) Individual probe intensity fold change for probeset not detected as deleted by probe intensity averaging.

**Fig. 9**

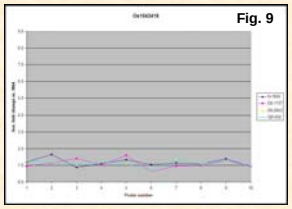


**Fig. 10**

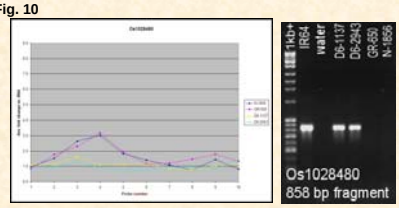



Figure 10 (left) a) Analysis of individual probe intensity fold change revealed deletions in part of the region covered by probeset Os1028480. This probeset was not detected as deleted by probe intensity averaging. b) PCR confirmation of deletion in GR-650 and N-1856. The position of this probeset is indicated in **green** in Fig. 7 and Table 1.

## Conclusions

- Development of *spl1* lesions on rice leaves is associated with up- and down-regulation of diverse genes, including putative defense response and signal transduction genes.
  - Ongoing comparisons of these patterns with those of other lesion mimic or defense response mutants will allow dissection of defense response pathways.
- The oligonucleotide array-based strategy is a rapid means to narrow candidates for the discovery of deleted genes.
  - The technique was most successful in detecting large deletions, e.g. those produced by radiation, because of the increased confidence afforded when more than one probe exhibited reduced intensity.
  - Using the allelic series of rice *spl1* mutants increased resolution and confidence.
  - Analysis of intensity from hybridization to individual probes in each probeset increased resolution and identified deletions that may be masked by averaging intensity values across the probeset.
  - The Rice GeneChip® arrays, designed for expression applications, are biased for probes or probesets in the 3' regions of genes. Ideally, for detection of deletions, probesets would sample full genes.

## Future studies

The validity of *spl1* candidates is being tested by expression knockdown using viral induced gene silencing (VIGS). Since the Syngenta Rice GeneChip® is proprietary, we are testing the publicly available Affymetrix rice arrays, which have a different design, to determine their utility in similar gene discovery assays.

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