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 aestivum, show enhanced resistance to pathogen invasion.

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.

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.

Conclusion

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 probes 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.

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.

Table 1

<table>
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<tr>
<th>Syngenta probeset</th>
<th>TIGR model ID</th>
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Acknowledgements

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