Transcript-Level Variation in Barley Seedling Leaves Challenged with Puccinia hordei and the Molecular Basis of Partial Resistance to Leaf Rust



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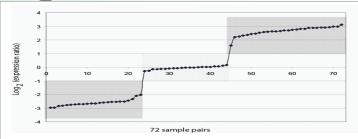
Microarray platform: 15k x 8 Barley 2 Agilent custom arrays

Introduction

In the present study, we employed an integrated strategy combining genetical genomics with genetic mapping to identify candidate genes for QTL previously identified for partial resistance to barley leaf rust. Genetical genomics is a novel strategy which uses transcript abundance as a surrogate phenotype (rust resistance) to map the genetic determinants for variation of gene expression, also known as expression QTL (eQTL) mapping. The advantage of the approach is that many thousands of surrogate phenotypes can be analyzed in a single experiment. eQTL mapping, when jointly analyzed with traditional phenotypic QTL analysis, offers considerable power to link observed transcript variation with phenotypic variation (leaf rust resistance). Co-localization of eQTL and phenotypic QTL can potentially help unravel the molecular basis of the observed phenotype and accelerate the identification of genes underlying partial resistance to leaf rust.

*60-mer probes *2 labelling colours Cy3 and Cy5 15208 genes (probes) per array A barley custom array was developed by incorporating 15208 genes onto a microarray made with the Agilent 2-channe platform. The custom array was used for RNA profiling on the 144 DH lines in 72 pairs of the Steptoe x Morex (St/Mx)

Allelic specific expression pattern

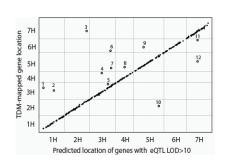


For genes with allelic expression pattern, Log expression ratio falls into three discrete classes depending on the genotypes of two paired samples. Dark grey boxes represent St/Mx or Mx/St, light grey box represents same genotypes either both of St or Mx. About 800 genes exhibited such expression pattern suggesting a cis regulatory fashion of the genes

eQTL location Expression QTL (eQTL) analysis identified 9557 genes with 1 to 6 significant eQTL (p<0.001) yielding 15685 eQTL in total. This figure gives an overview of eQTL mapping results by plotting eQTL positic against target gene positions previously mapped by SNP and TDM (transcription derived markers)

Overview of eQTL results

Gene locations are predictable on eQTL LOD scores and highly consistent with TDM



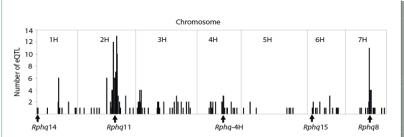
		eQTL-	locat		locati		
	Unigene#	linked SNP	Chr. cM			Chr. cM	
1	16599	2_0502	1H	4	3H	175	
2	6781	1_1267	1H	100	3H	133	
3	15402	1_0625	2H	206	7H	108	
4	26527	2_0115	3H	111	4H	153	
5	19223	2_0944	3H	169	3H	226	
6	1579	2_0085	3H	191	6H	3	
7	20765	2_0920	3H	196	5H	45	
8	11213	1_0046	4H	86	5H	56	
9	12083	1_0058	5H	77	6H	55	
10	4350	1_1092	5H	215	2H	142	
11	21295	1_0055	7H	101	6H	158	
12	4267	1 0055	7H	101	5H	140	

Positional comparison of eQTL with target genes that are mapped by SNP revealed that eQTL with LOD>10 were exclusively mapped within 10 cM to their target genes. Thus LOD>10 was used as a criteria to predict gene locations previously not mapped which allowed for comparison of predicted locations with those mapped by TDM. Of 553TDM-mapped genes, 40 genes (7%) showed discrepancy exceeding 10 cM from predicted positions of which 12 genes (represent by the empty numbered circles with details attached in the table) showed distinct difference.

Conclusion remarks

By using strategy of genetical genomics in combination with resistance QTL mapping, a number of candidate genes have been identified. Further investigation such as genetic or comparative mapping of candidate genes with rice genome will further refined the candidate gene list. And in addition, ontology analysis of genes correlated with rust resistance and genes with eQTL co-localized with resistance QTL may point to master regulators and regulatory pathways underlying the partial resistance. Knowledge of the genes regulated by the master regulators may provide insight into the molecular and biochemical nature of the resistance, and help cloning the causal genes.

Distribution of eQTL for genes with expression correlated with rust resistance



Correlation analysis identified 128 gene significantly correlated with rust resistance. The figure shows the distribution of eQTL for these genes across the genome. Most notably, eQTL for the top 24 genes were exclusively co-localised with Rphq11, the QTL with largest effect for partial resistance to leaf rust

Identification of candidate genes on regulatory fashion

Resistance QTL	eQTL	cis	trans	unknown
Rphq14 (1H)	1	1	0	0
Rphq11 (2H)	54	32	2	20
Rphq-4H (4H)	8	4	0	4
Rphq15 (6H)	4	3	1	0
Rphq8 (7H)	26	9	2	15
total	93	49	5	39

Regulatory fashion of eQTL co-localised with the resistance QTLs. Those of genes being cis-regulated are priority candidates, while genes with as yet unknown regulatory fashion are potential candidates for resistance to leaf rust

Acknowledgements

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