

# Genetic Factors Associated with Tolerance of Zinc Deficiency in Rice

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## INTRODUCTION

Zinc deficiency is the most widespread micronutrient disorder in rice (*Oryza sativa* L.). Efforts to develop cultivars with improved tolerance to Zn deficiency have been hampered by insufficient understanding of genetic factors contributing to it. The objectives of this study were to map quantitative trait loci (QTL) associated with tolerance to Zn deficiency under field conditions and to follow up with a micro-array experiment for further transcriptomic profiling of two contrasting genotypes.

## METHODS

The QTL mapping was done with 110 recombinant inbred lines (RIL) derived from the cross of the intolerant parent IR74 with the tolerant donor Jalmagna. This population was phenotyped in a highly Zn deficient field and a fertilized control plot using plant mortality, leaf bronzing and shoot dry matter as indicators of Zn deficiency responses (Wissuwa et al. 2006). The RIL-46 was selected from the mapped population for further studies because it contained tolerance alleles at all influential QTL. The variation in gene expression patterns between RIL-46 and its parent IR74 in response to Zn deficiency was investigated using the Affymetrix GeneChip. Ribonucleic acid was extracted for this micro-array work from roots and leaves of field grown plants sampled four weeks after transplanting when symptoms of Zn deficiency began to appear.

## RESULTS AND DISCUSSION

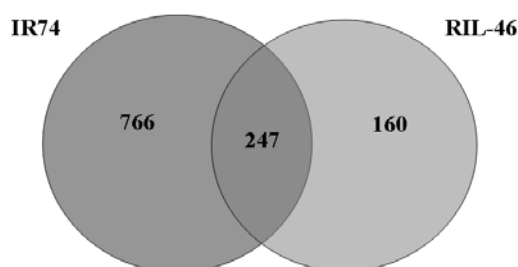
Lines grown in the Zn deficient field plot showed reductions in Zn content and dry matter of 90% and 80%, respectively, relative to the control. The most severe symptom of Zn deficiency was plant mortality reaching up to 100%. Leaf bronzing, as susceptibility indicator, did not necessarily concur with high mortality, which implied that both traits were under independent genetic control. The QTL mapping experiment largely confirmed this since only one minor QTL for mortality co-localized with QTL detected for leaf bronzing (Table 1). Four QTL associated with mortality were detected. The two most influential QTL were on chromosomes 2 and 12, and alleles of the tolerant donor parent Jalmagna reduced mortality by 16.6 and 14.8%, respectively. The most important QTL for leaf bronzing was on chromosome 4, and Jalmagna alleles reduced bronzing by 30%. The QTL for mortality acted in a purely additive manner whereas digenic epistatic interactions involving the main QTL for mortality on chromosome 12 were important for leaf bronzing and dry matter.

Results of the micro-array experiment showed that Zn deficiency led to an up-regulation of more genes in IR74 compared to RIL-46 (Fig. 1). Among the common up-regulated genes, the degree of up-regulation in IR74 was higher for several genes potentially involved in Zn uptake and translocation such as Zn transporters and ZIP family proteins. This pattern

suggested that the strong response of IR74 was a reflection of a higher stress experienced by IR74 and that other genes caused a higher tolerance of RIL-46.

**Table 1. The quantitative trait loci (QTL) for plant mortality and leaf bronzing detected in a field trial in a Zn deficient soil.**

Chromo some	Marker Interval (epistatic interaction)	QTL	Position (cM)	LOD	R <sup>2</sup>	Allelic effect (%)	Positive allele
<b>Plant Mortality</b>							
1	<u>RG109</u> - Sd1	<i>Zmt1</i>	138	2.9	11.8	+9.8	IR74
2	P2M9-8 - <u>P2M6-8</u>	<i>Zmt2</i>	42	4.2	16.6	-16.6	Jalmagna
7	P2M7-7 - <u>P2M6-5</u>	<i>Zmt7</i>	20	2.7	11.3	-9.2	Jalmagna
12	CDO344-1 - <u>RG543-1</u>	<i>Zmt12</i>	150	6.4	24.2	-14.8	Jalmagna
<b>Leaf bronzing</b>							
1	<u>P1M10-14</u> - P2M10-11	<i>Zbz1a</i>	80	4.4	17.8	+1.64	IR 74
1	<u>RG220</u> - RG109	<i>Zbz1b</i>	124	4.1	16.5	+1.20	IR 74
4	<u>P3M1-5</u> - P3M5-1	<i>Zbz4</i>	92	4.7	18.7	-1.84	Jalmagna
12	<u>P3M5-9</u> - P1M7-4	<i>Zbz12</i>	70	3.6	14.6	+0.92	IR 74
12 x 9	( <i>Zmt12</i> x C9/115cM)				5.7		Jal x IR74
12 x 3	( <i>Zmt12</i> x C3/125cM)				12.4		Jal x IR74



**Fig. 1. Probe sets up regulated by Zinc deficiency. Among these a Zn transporter (Os05g39560.1) was up regulated 100-fold in IR74 but only 25-fold in RIL-46.**

## CONCLUSIONS

The identified *Zmt12* can be considered a key factor for tolerance to Zn deficiency among the QTL. The *Zmt12* explains a major portion of the variation for mortality, and it is also involved in several epistatic interactions for leaf bronzing and dry matter accumulation. These epistatic interactions involving mostly IR74 alleles in combination with the Jalmagna allele at *Zmt12* may largely explain the transgressive segregation observed for all traits evaluated (Wissuwa et al. 2006). One potential mechanism for the superior tolerance of several RILs of the mapping population is their higher rate of citrate excretion from roots under Zn deficiency compared to IR74 (Hoffland et al. 2006). Additional tolerance mechanisms will be discussed with particular reference to the transcriptome analysis of the two contrasting genotypes IR74 and RIL-46.

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