

# A genome wide association study (GWAS) to identify genes associated with tolerance to iron toxicity in rice (*Oryza sativa* L.)

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## ABSTRACT

Iron ( $\text{Fe}^{2+}$ ) toxicity limits rice (*Oryza sativa* L.) growth in paddy fields worldwide, reducing yields up to complete crop failure<sup>1</sup>.  $\text{Fe}^{2+}$  leads to the formation of reactive oxygen species (ROS) within the plant, followed by typical bronzing of the leaves<sup>2</sup>. Previous work aimed at genetically dissecting this complex trait has focused mainly on QTL approaches using bi-parental mapping populations, thereby covering only a narrow genetic basis. Here we report a genome wide association study (GWAS), identifying iron tolerance loci in a panel of 329 varieties, representing all sub-groups of *O. sativa*<sup>3</sup>. The lines were grown in hydroponic solutions in the green house for four weeks and then exposed to an iron pulse stress of 1000 ppm  $\text{Fe}^{2+}$  for five days. All plants were phenotyped by quantifying leaf damage and growth. *Temperate japonica* and *aromatic* sub-groups proved more tolerant than *tropical japonica*, *indica* and *aus* ( $p < 0,001$ ). SNP markers significantly associated with iron tolerance were located near or inside potential candidate genes predicted or demonstrated to function in metal transport and detoxification. Currently, a subset of 32 selected lines contrasting in iron tolerance is being validated under field conditions on an iron toxic acid sulfate soil in Iloilo, Philippines. This project will add to the genetic understanding of iron uptake, transport and tolerance mechanisms, which is the basis for enhancing yield and producing fortified crops on iron toxic soils.

## REFERENCES

<sup>1</sup>Abifarin, A. O. (1988) Grain yield loss due to iron toxicity. West Africa Rice Development Association (WARDA) *Technical Newsletter* **8**, 1-2

<sup>2</sup>Becker, M., Asch, F. (2005) Iron toxicity in rice — conditions and management concepts. *Journal of Plant Nutrition and Soil Science* **168**, 558–573.

<sup>3</sup>Zhao, K., Tung, C.-W., Eizenga, G. C., Wright, M. H., Ali, M. L., Price, A. H., Norton, G. J., *et al.* (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nature communications* **2**:467

# A genome-wide association study (GWAS) to identify genes associated with tolerance of iron toxicity in rice (*Oryza sativa* L.)



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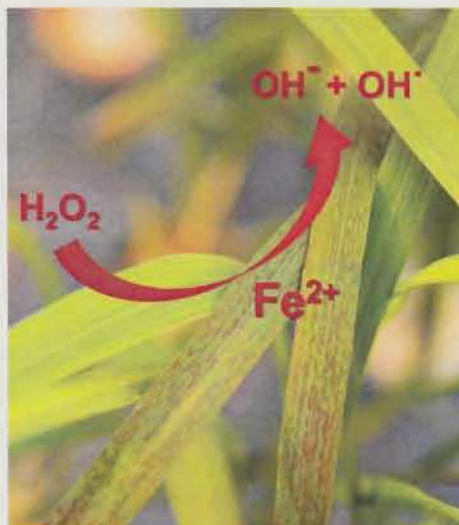


## The problem

Three-quarters of all rice is produced under anoxic lowland conditions. In aerated soils, the essential micronutrient iron is mostly complexed and unavailable to plants. Upon submergence, iron is reduced to its soluble form  $Fe^{2+}$ , which can be taken up in excess. Iron toxicity leads to the generation of reactive oxygen species (ROS) and causes leaf bronzing, yield reduction, or complete crop failure, especially in West Africa and Southeast Asia.

Different tolerance mechanisms have been proposed—including iron exclusion, compartmentalization, and detoxification—but the genetic basis of these mechanisms remains unknown.

The aim of this project is the identification of candidate genes and gene networks contributing to iron toxicity tolerance. Understanding of iron metabolism in rice is essential for both biofortification and the breeding of tolerant varieties for problem soils.



## The material

Asian rice offers vast genetic diversity because of its long domestication history.

A 'diversity panel' was used for GWAS:

- 329 diverse *O. sativa* genotypes from 79 countries
- All five subpopulations were represented
- It was previously genotyped for more than 44,000 single-nucleotide polymorphisms (SNPs) by Zhao *et al.* (2011).
- Genotype data were publicly available at [www.ricediversity.org](http://www.ricediversity.org)



Diversity of Asian rice

## Approach I: GWAS



Plants were grown in hydroponic culture and exposed to an iron pulse stress of 1000 ppm  $Fe^{2+}$  for five days during the vegetative growth stage.

To avoid oxidation and precipitation of iron, nutrient solutions were bubbled with nitrogen at 2-hour intervals.

Leaf bronzing was scored at 3 and 5 days after start of treatment. Biomass and plant growth were recorded.

The hydroponic system with frequent  $N_2$  percolation simulated a high iron pulse stress at the greenhouses of University of Bonn in Germany.

## Conclusions and outlook

Subpopulations *indica*, *aus*, and *tropical japonica* were the most susceptible to iron toxicity.

GWAS led to the identification of SNPs significantly linked to iron tolerance. Haplotype analysis is ongoing to identify candidate genes involved in iron homeostasis.

A field trial of selected lines showed genotype-dependent patterns of response to iron toxicity, suggesting different underlying genetic mechanisms. Evaluation of yield under iron stress is ongoing.

Combining data from GWAS and from the field trial will form the basis for elucidating gene (networks) important for iron homeostasis and detoxification.

## Approach II: field validation

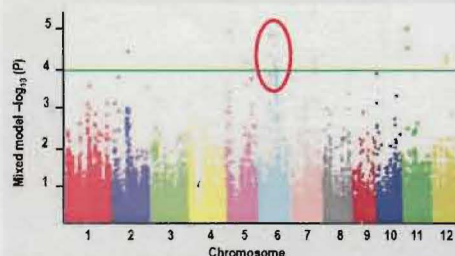
A subset of 32 genotypes, differing in iron tolerance, is currently being tested on an iron-toxic acid-sulfate soil in Ajuy, Philippines, in cooperation with IRRI.

Plants are phenotyped for leaf bronzing, growth parameters, and yield under stress and control conditions.



Constant iron stress conditions on a field in Ajuy, Iloilo, Philippines.

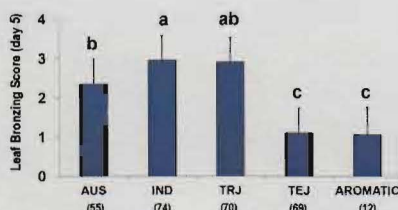
## Results I



SNPs significantly associated with iron toxicity response, based on leaf bronzing scored after three days of stress (significance threshold  $p < 0.0001$ ; green line). Encircled region contains significant SNPs located in a genomic region rich in peroxidases.

Associating genotypic and phenotypic data using a mixed linear model localized SNPs significantly associated with iron toxicity response, e.g., peroxidases, which are known to function in the detoxification of ROS.

## Results II



Mean leaf bronzing score (0 = no symptoms; 2 = brown spots appear on tips of leaves; 4 = brown spots affect whole leaves, and leaves start to discolor) of subpopulations *aus*, *indica*, *tropical japonica*, *temperate japonica*, and *aromatic* after 5 days of 1000 ppm  $Fe^{2+}$  pulse stress (hydroponics). Given in parentheses are the number of lines, with  $n$  per line = 4. Bars depict standard deviation.

Leaf bronzing score differed significantly among subpopulations after a 5-day iron pulse stress.

## Results III

Designation	Subpop.	Field site			Greenhouse
		4 weeks Constant medium iron	8 weeks Constant low iron	8 weeks Constant medium iron	5 days Pulse high iron
Dom Sufid	<i>aro</i>	t	t	t	t
Bico Branco	<i>aro</i>	t	t	t	t
Djimonon	<i>ind</i>	s	s	s	s
Gogo Lempuk	<i>trj</i>	s	s	s	s
Dular	<i>aus</i>	s	s	s	t
Sadu Cho	<i>ind</i>	s	s	s	t
DV85	<i>aus</i>	t	t	t	s
RTS 4	<i>ind</i>	t	t	t	s

Selected lines from the diversity panel showed different patterns of iron toxicity response (t = tolerant; s = susceptible) under varying stress conditions.

Genotype-dependent tolerance patterns suggest different mechanisms of tolerance to iron toxicity. Haplotype analysis to dissect underlying genetic basis of tolerance is ongoing.

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