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 (Fe²⁺) toxicity limits rice (Oryza sativa L.) growth in paddy fields worldwide, reducing yields up to complete crop failure¹. Fe²⁺ leads to the formation of reactive oxygen species (ROS) within the plant, followed by typical bronzing of the leaves². 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. sativa³. The lines were grown in hydroponic solutions in the greenhouse for four weeks and then exposed to an iron pulse stress of 1000 ppm Fe²⁺ 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


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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 volatile form Fe(II), 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.

Approach I: GWAS

Plants were grown in hydroponic culture and exposed to an iron pulse stress of 1000 ppm Fe(II) for five days during the vegetative growth stage. To avoid oxidation and precipitation of iron, nutrient solutions were bubbled with nitrogen at 2-hr intervals. Leaf bronzing was scored at 3 and 5 days after start of treatment. Biomass and plant growth were recorded.

Conclusions and outlook

Subpopulations indica, aux, and tropical japonica were the most susceptible to iron toxicity. GWAS led to the identification of SNPs significantly linked to iron tolerance. Haplotypes 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 that are 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 Auy, Philippines, in cooperation with IRRI.

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

Results I

Results II

Results III

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 distortion) of subpopulations aux, indica, tropical japonica, temperate japonica, and refrigerated after 5 days of 1000 ppm Fe(II) pulse stress (hydroponics). Given in parentheses are the number of lines, with n per line × 4. Bars depict standard deviation.

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

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 is expected to reveal the genetic basis of tolerance to ongoing.