GENETIC APPROACHES TO ADAPT RICE PRODUCTION TO IRON TOXICITY

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Iron Toxicity at Low Redox Potential

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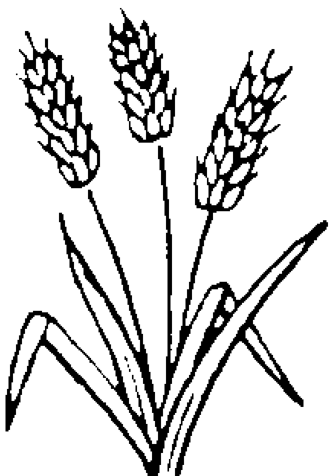
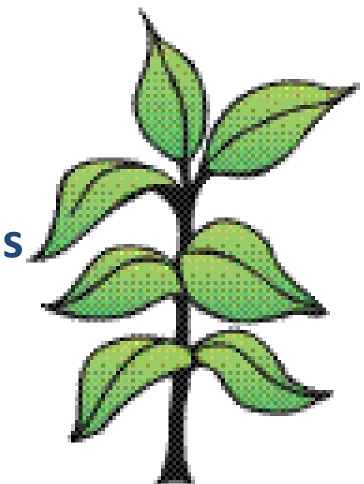
Graminaceae

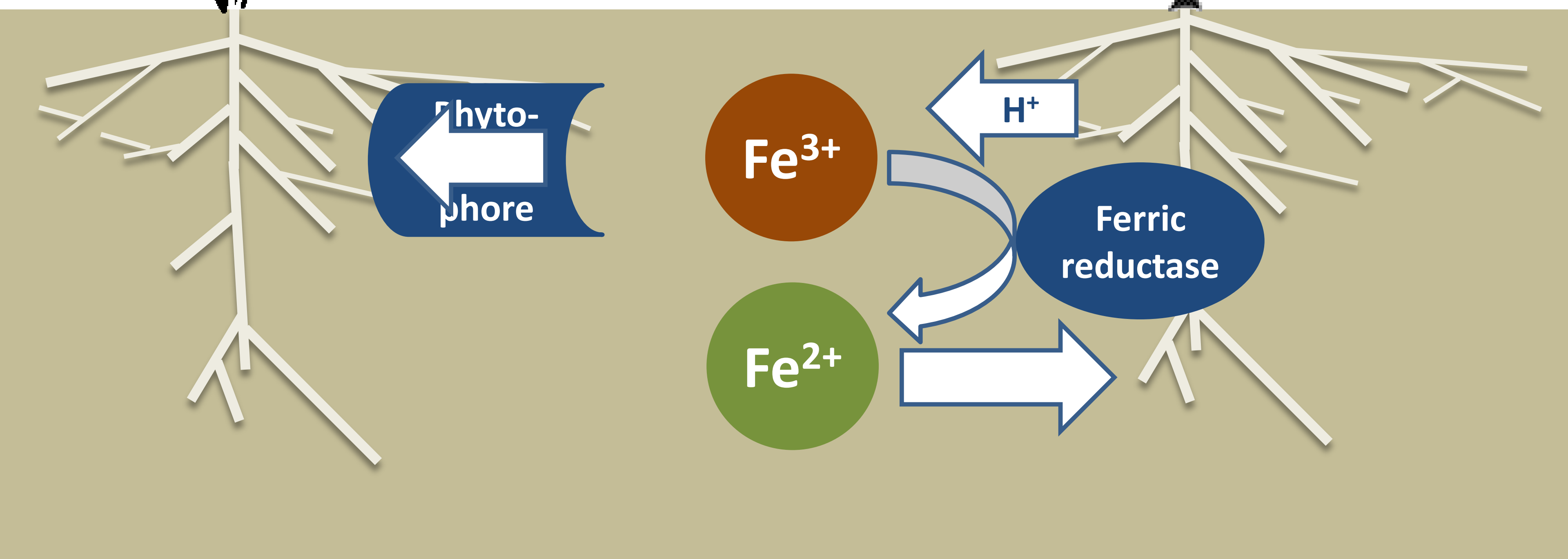


Non-Graminaceae



Functions of Fe in plants:

- Involved in electron transport chains
- Component of many enzymes
- Involved in chlorophyll synthesis



Iron Toxicity at Low Redox Potential

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Prevalent microbial metabolism

Soil redox potential Eh (mV)

Aerobic (O_2 → H_2O)

+200 — Nitrate reduction (NO^{3-} → N_2, NH_4^+)

Manganese reduction (Mn^{4+} → Mn^{2+})

Iron reduction (Fe^{3+} → Fe^{2+})

0 —

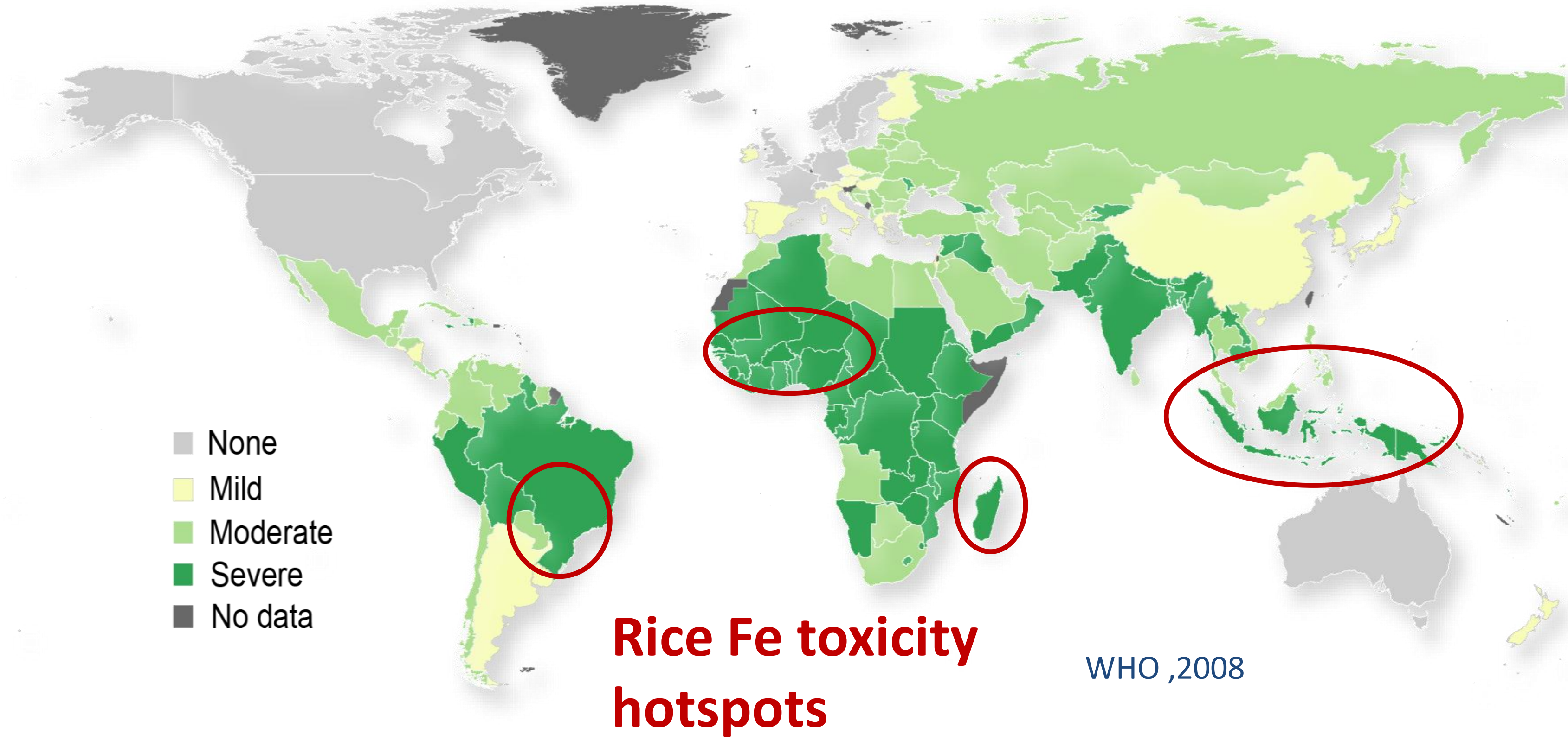
Sulfate reduction (SO_4^{2-} → H_2S)

-200 — Methanogenesis (CO_2 → CH_4)

Iron Toxicity in Rio Grande do Sul

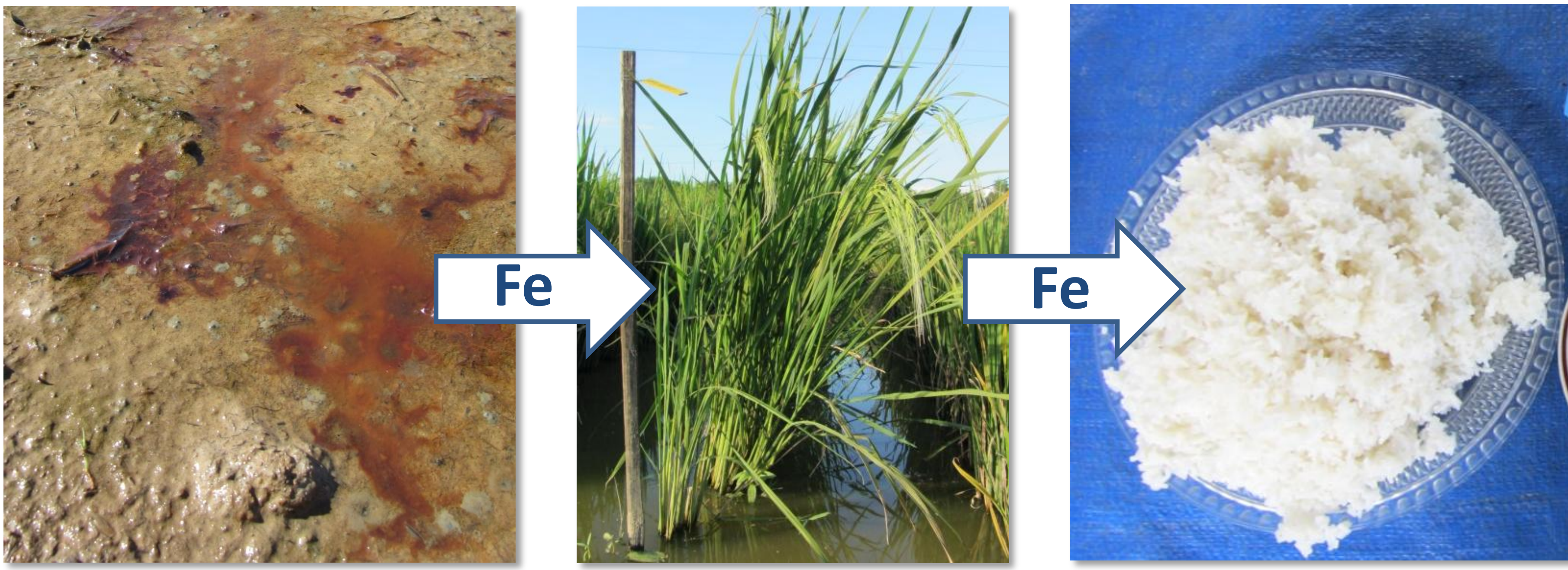
Photos: Sergio Lopez, IRGA

Iron Deficiency in Human Diets



- More than 2 billion people worldwide lack sufficient Fe in their diets
- Human Fe deficiency often coincides with Fe toxicity in rice

Aims of this Project



To identify genes and mechanisms associated with tolerance to high Fe soils

To maximize the Fe translocation from high Fe soils to the grain

Research Questions

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1. Is there genetic variation we can use for adaptation and what are the underlying mechanisms?

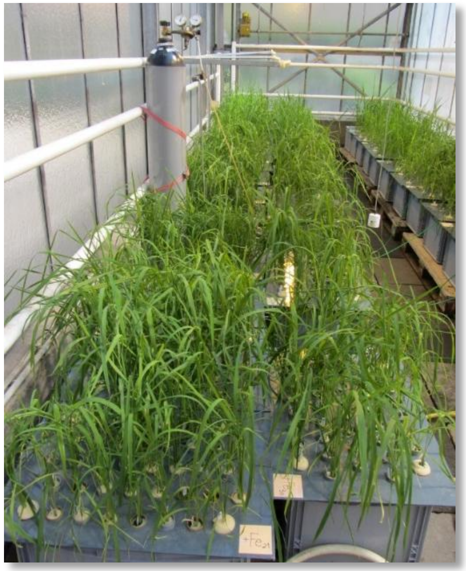
2. Can we use rice wild relatives for adaptation to Fe toxicity?

3. Do tolerant varieties accumulate more Fe in their grains than intolerant ones?

Research Questions

1. Is there genetic variation we can use for adaptation and what are the underlying mechanisms?

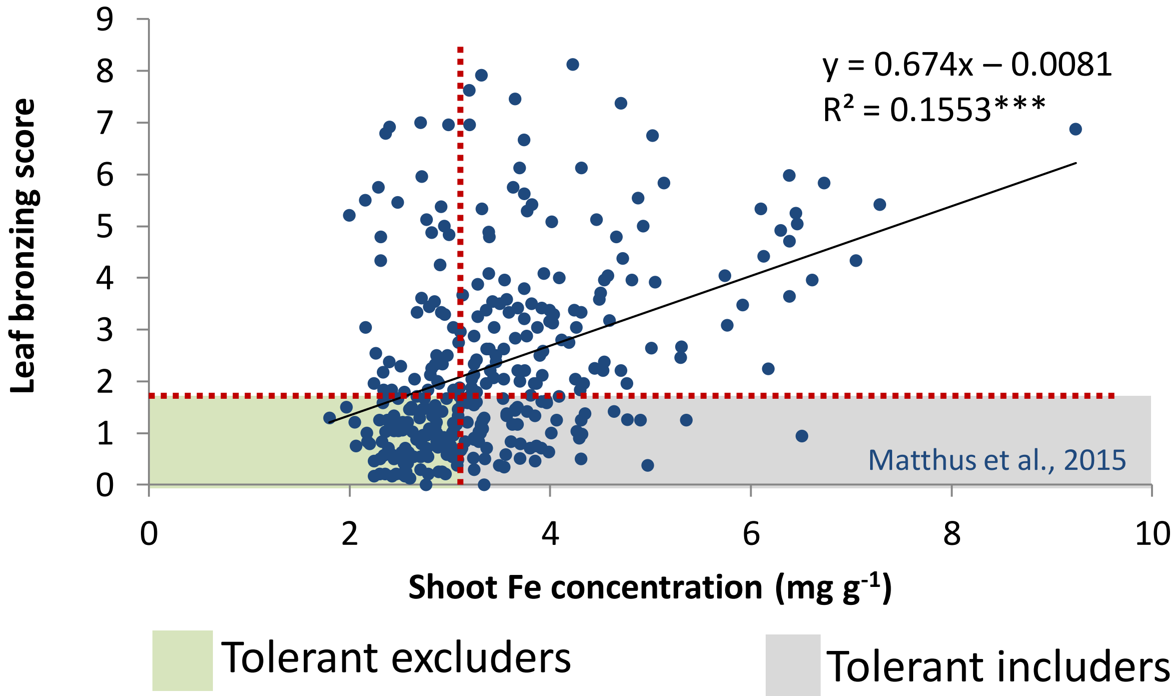
Hydroponic Screening Experiments



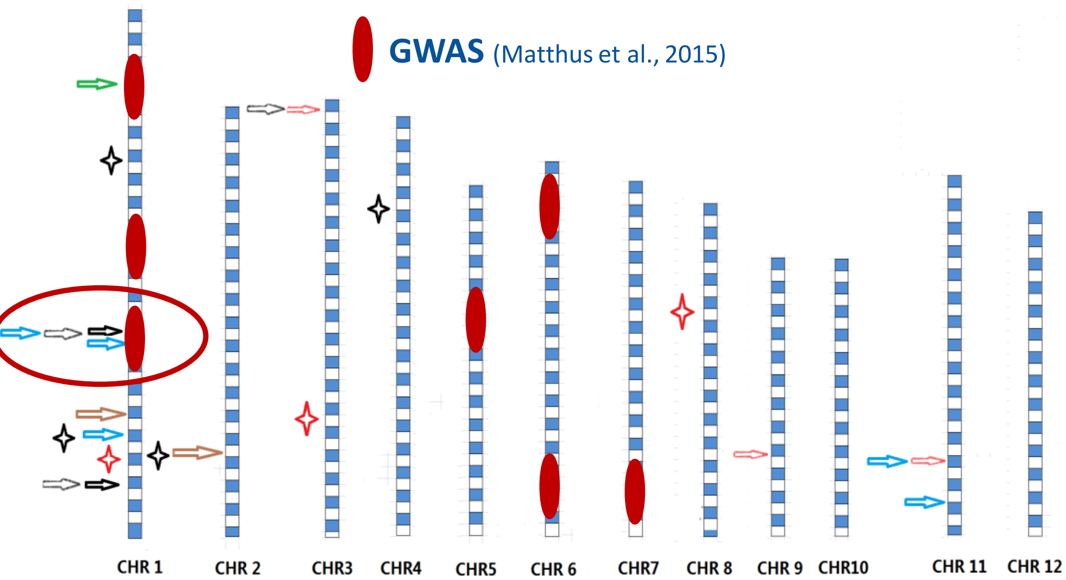
Leaf bronzing score

Wu et al., 2014

Shoot Fe concentration vs. leaf symptoms



QTL for Fe Tolerance

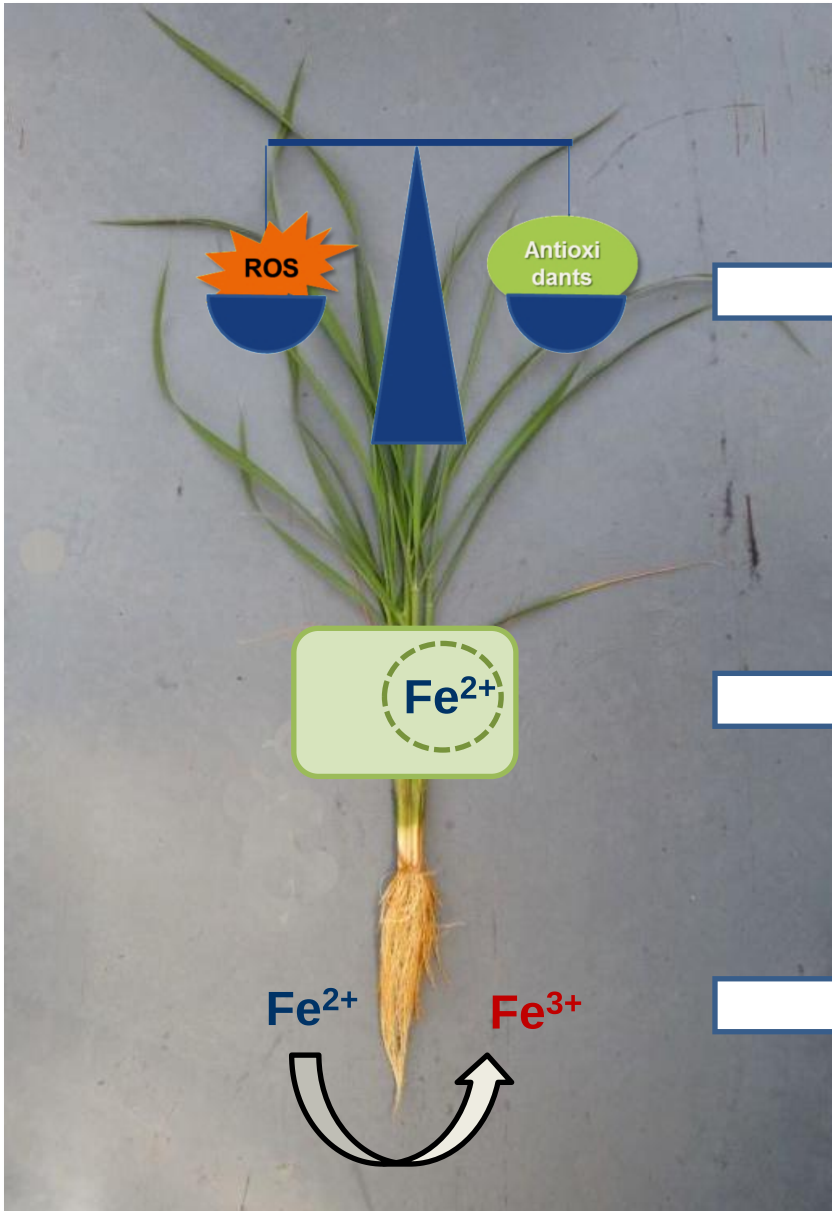


Wu et al. (2014)

Integrated genetic map of Fe tolerane QTL

- Many small effect loci
- Highly dependent on environment and stress type
- Some conserved regions

Tolerance Mechanisms



Redox homeostasis

Iron partitioning

Exclusion at the root surface

Genotypes Contrasting in Shoot Tolerance

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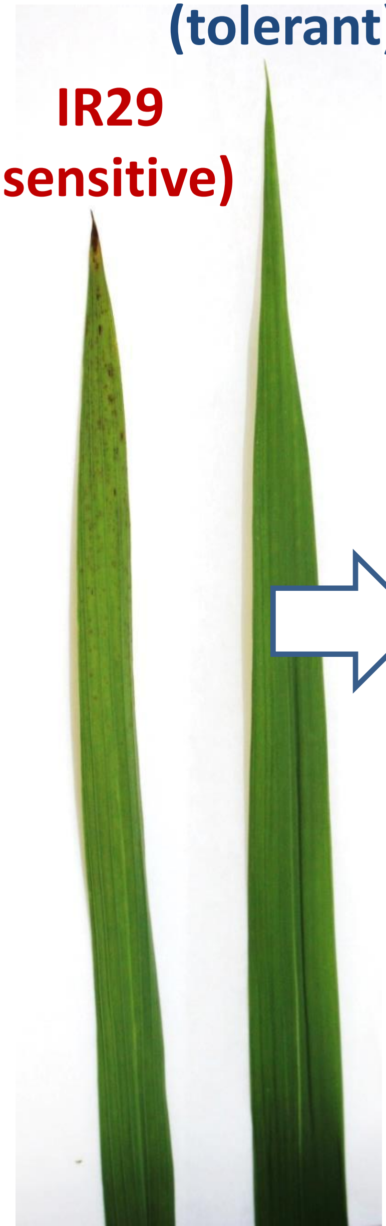
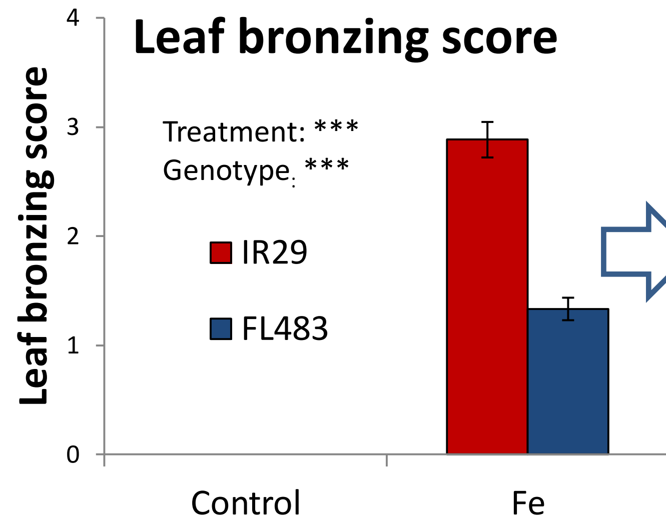
FL483 (tolerant)

IR29 (sensitive)

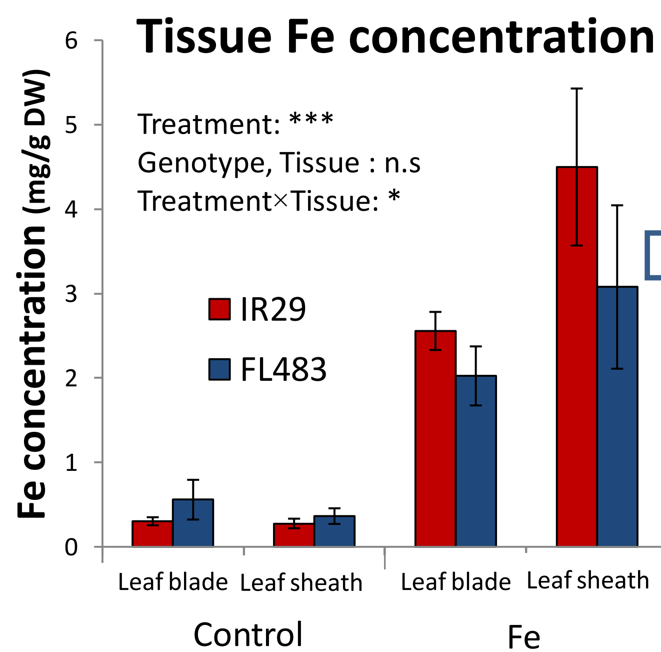


Carries positive alleles at two QTL in IR29 genetic background

(Wu et al., 2014)

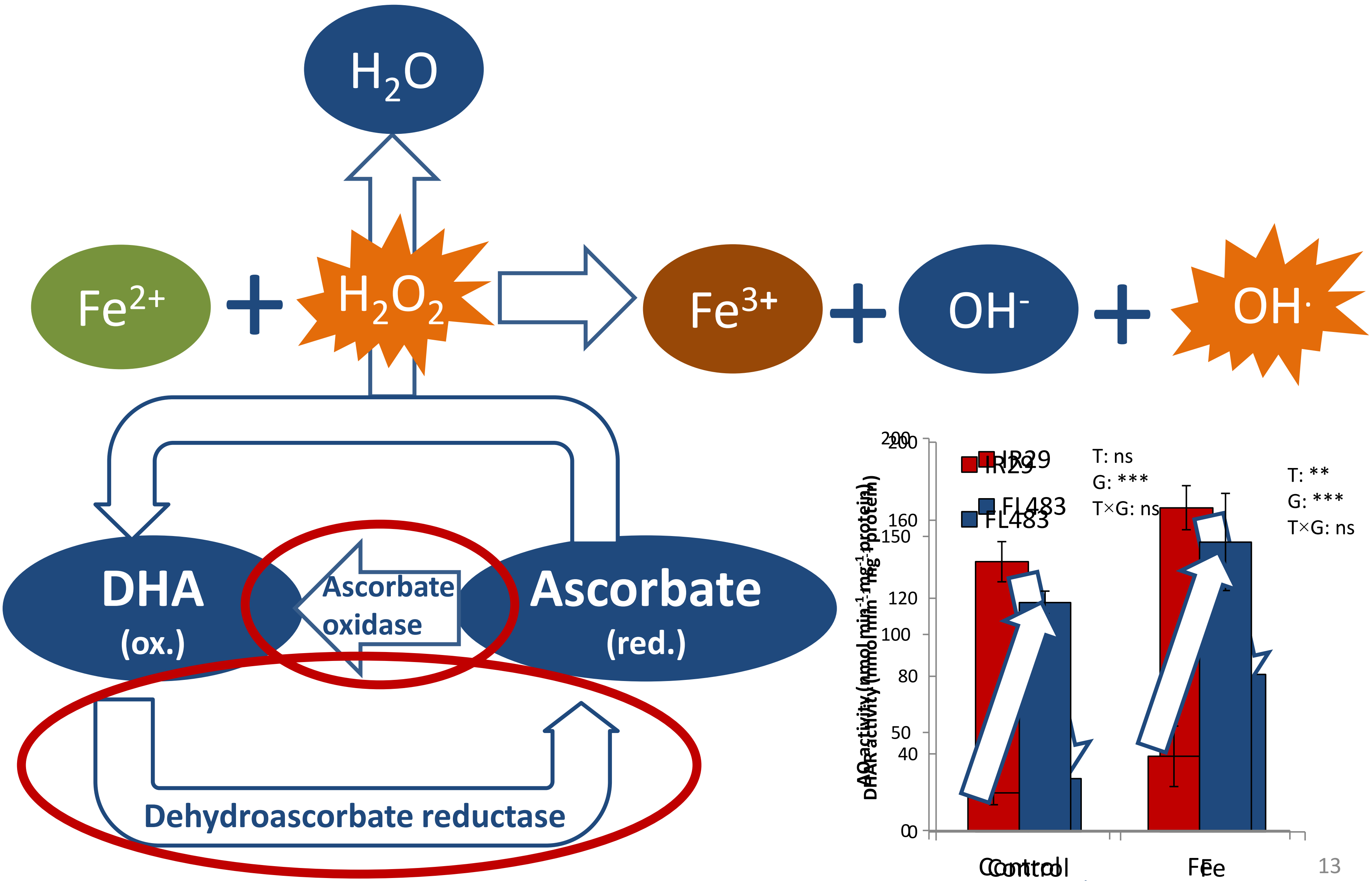


Less symptoms in FL483



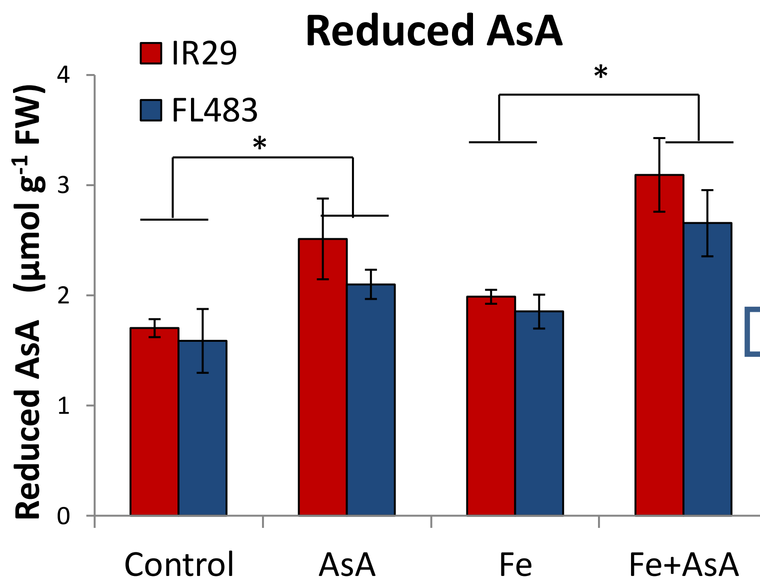
No difference in shoot Fe concen-tration

Antioxidants and Fenton Chemistry



Wu et al., 2017

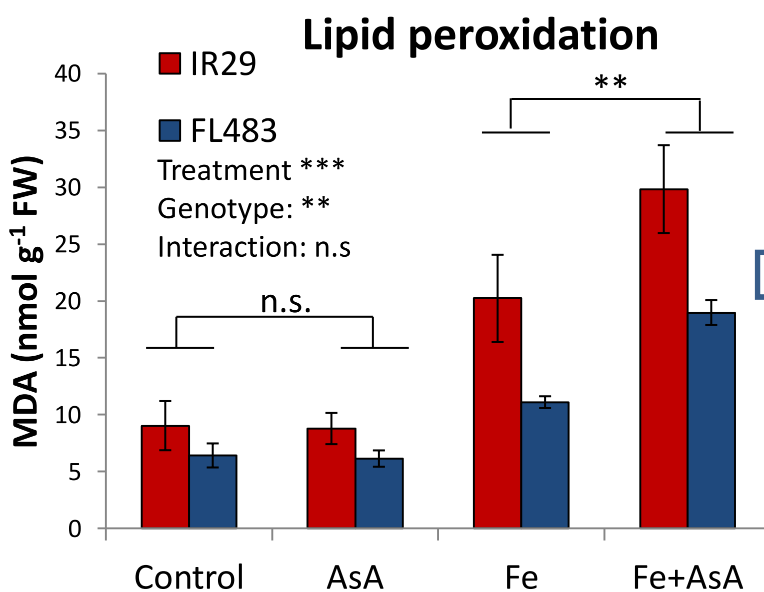
Exogenous Ascorbate Application



Increase in foliar AsA

Four treatments:

1. Control
2. Control + exogenous AsA (5mM foliar spray)
3. Fe toxicity
4. Fe toxicity + exogenous AsA



Increase in lipid per-oxidation

Wu et al., 2017

Exogenous Ascorbate Precursor Application

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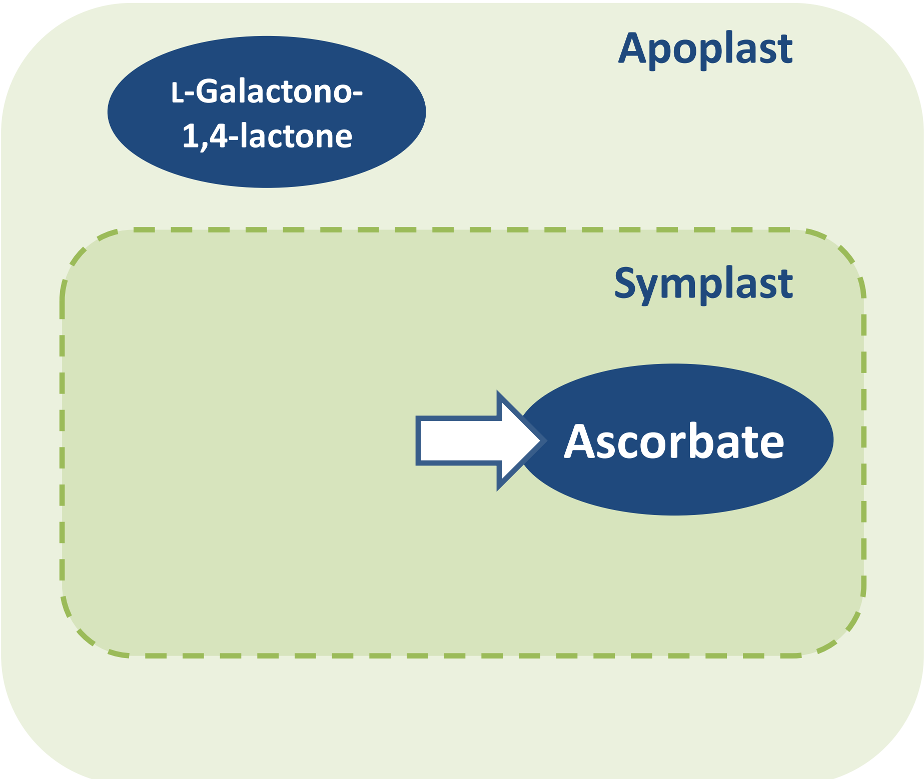
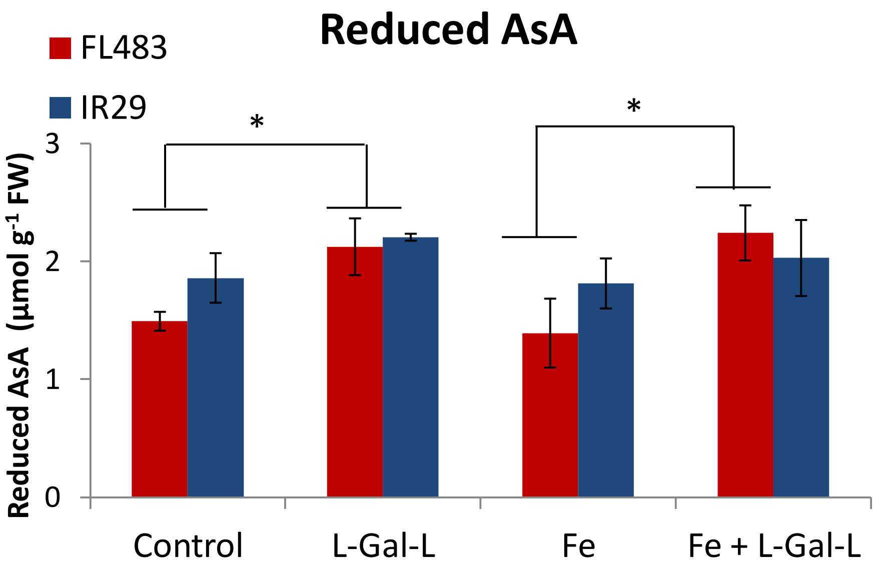
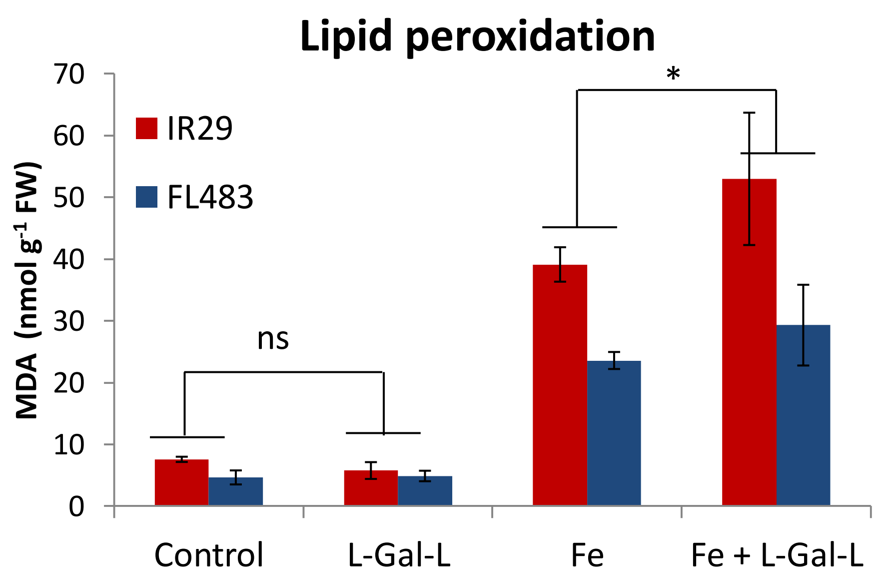


Four treatments:

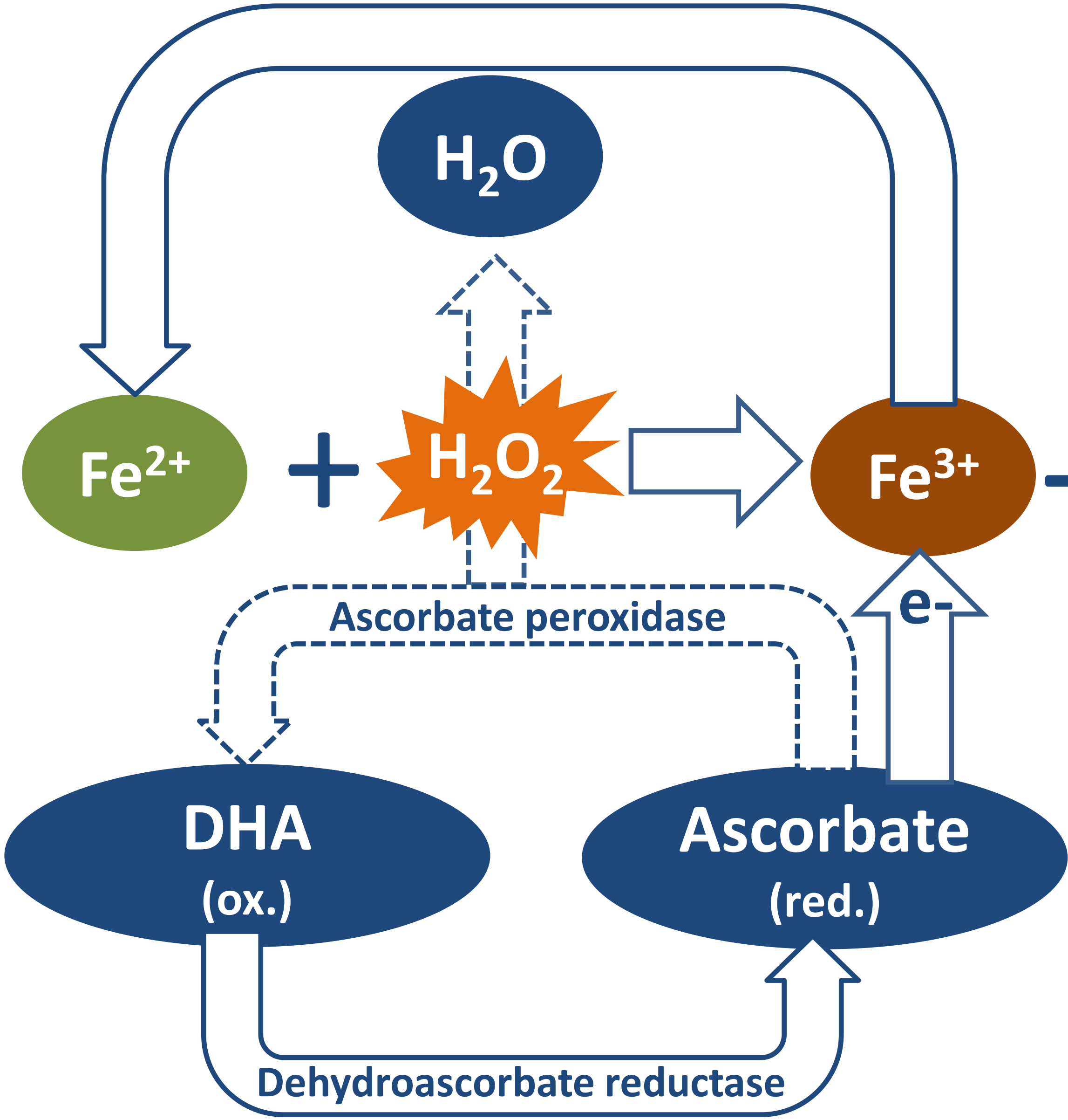
1. Control
2. Control + exogenous L-Galactono-1,4-lactone (L-Gal-L)
3. Fe toxicity
4. Fe toxicity + exogenous L-Gal-L

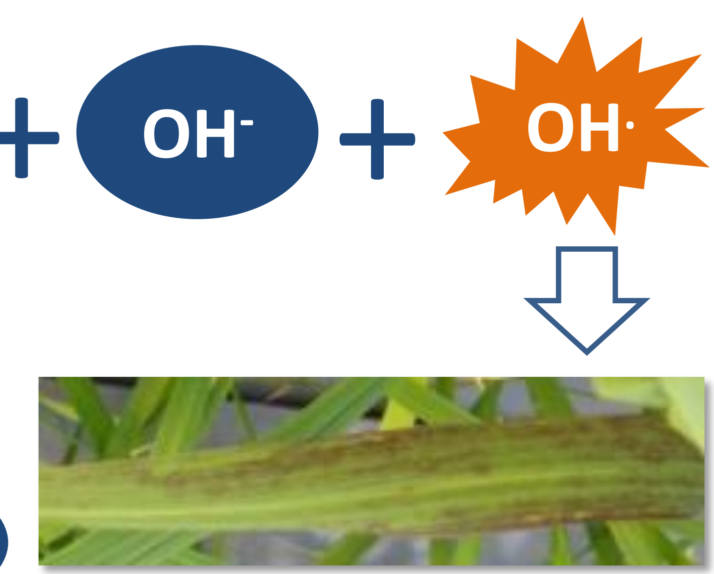




Wu et al., 2017

Ascorbate Stimulates the Fenton Reaction





Keeping AsA turnover low confers tolerance!

Wu et al., 2017

Ionomic Profile of *OsAKT1* Mutants

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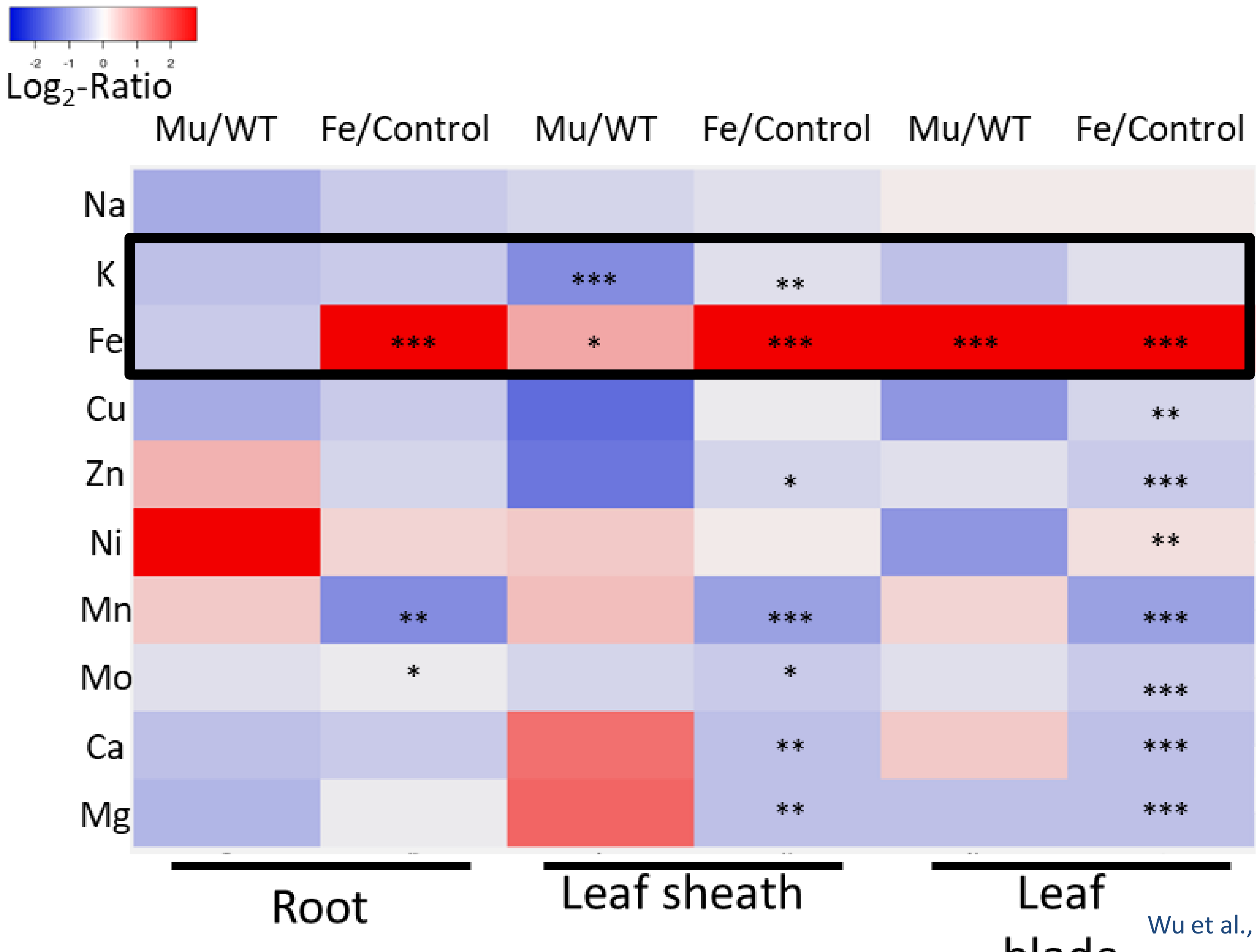
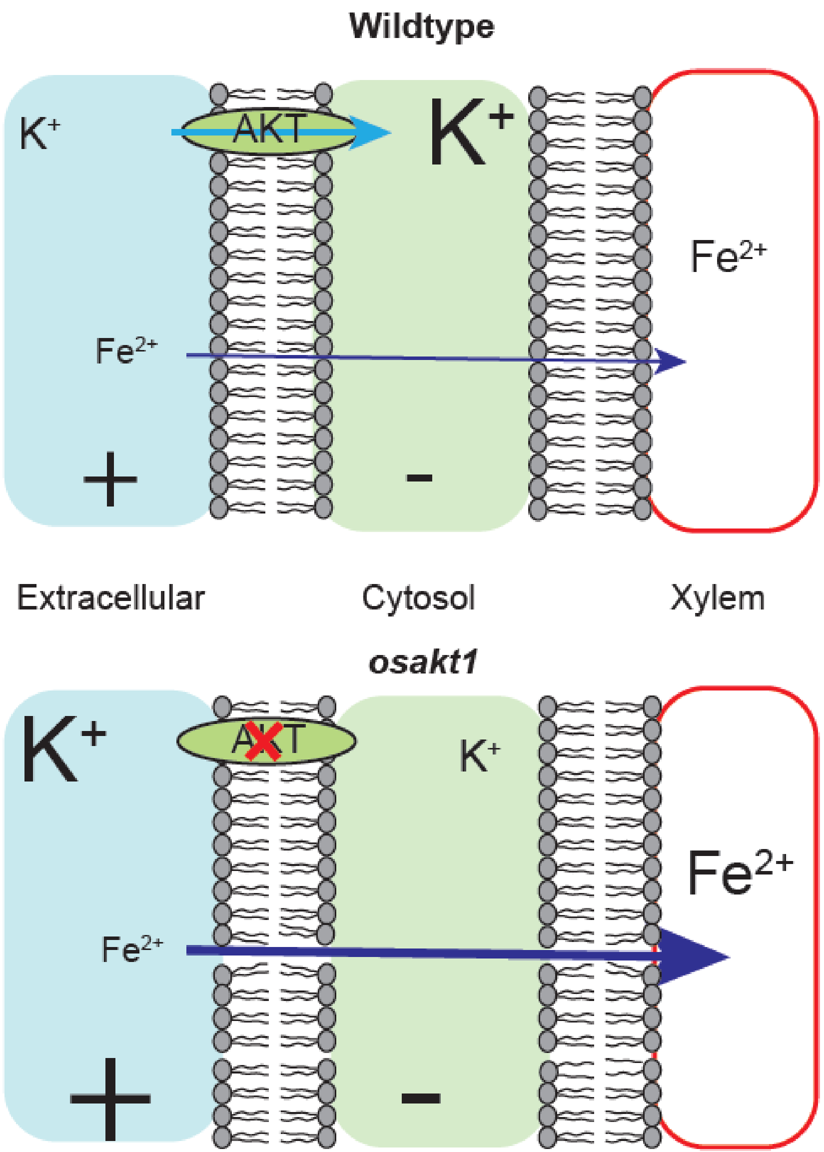


Wu et al., 2019

More Fe and less K in the KO-mutants

Mechanistic Model of Fe-Exclusion



Plant maintains less polarized membrane potential

Hyperpolarization of the membrane falilitates Fe-influx

Wu et al., 2019

Research Questions

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1. Is there genetic variation we can use for adaptation and what are the underlying mechanisms?

- Yes, both exclusion and inclusion mechanisms can be found in the natural gene pool of rice.
- Shoot tolerance related to ascorbate recycling
- Exclusion related to potassium uptake

Research Questions

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2. Can we use rice wild relatives for adaptation to Fe toxicity?

Genome Sequences for Rice Wild Relatives

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nature genetics

ARTICLES

https://doi.org/10.1038/s41588-018-0040-0

Corrected: Publisher Correction OPEN

Genomes of 13 domesticated and wild rice relatives highlight genetic conservation, turnover and innovation across the genus *Oryza*

Joshua C. Stein[1], Yeisoo Yu[2,21], Dario Copetti[2,3], Derrick J. Zwickl[4], Li Zhang[5], Chengjun Zhang[5], Kapeel Chougule[1,2], Dongying Gao[6], Aiko Iwata[6], Jose Luis Goicoechea[2], Sharon Wei[1], Jun Wang[7], Yi Liao[8], Muhua Wang[2,22], Julie Jacquemin[2,23], Claude Becker[9], Dave Kudrna[2], Jianwei Zhang[2], Carlos E. M. Londono[2], Xiang Song[2], Seunghee Lee[2], Paul Sanchez[2,24], Andrea Zuccolo[2,25], Jetty S. S. Ammiraju[2,26], Jayson Talag[2], Ann Danowitz[2], Luis F. Rivera[2,27], Andrea R. Gschwend[5], Christos Noutsos[1], Cheng-chieh Wu[10,11], Shu-min Kao[10,28], Jhih-wun Zeng[10], Fu-jin Wei[10,29], Qiang Zhao[12], Qi Feng[12], Moaine El Baidouri[13], Marie-Christine Carpentier[13], Eric Lasserre[13], Richard Cooke[13], Daniel da Rosa Farias[14], Luciano Carlos da Maia[14], Railson S. dos Santos[14], Kevin G. Nyberg[15], Kenneth L. McNally[3], Ramil Mauleon[3], Nickolai Alexandrov[3], Jeremy Schmutz[16], Dave Flowers[16], Chuanzhu Fan[7], Detlef Weigel[9], Kshirod K. Jena[3], Thomas Wicker[17], Mingsheng Chen[8], Bin Han[12], Robert Henry[18], Yue-ie C. Hsing[10], Nori Kurata[19], Antonio Costa de Oliveira[14], Olivier Panaud[13], Scott A. Jackson[6], Carlos A. Machado[15], Michael J. Sanderson[4], Manyuan Long[5], Doreen Ware[1,20] and Rod A. Wing[2,3,4*]

Expanding the Gene Pool: Screening Wild Relatives

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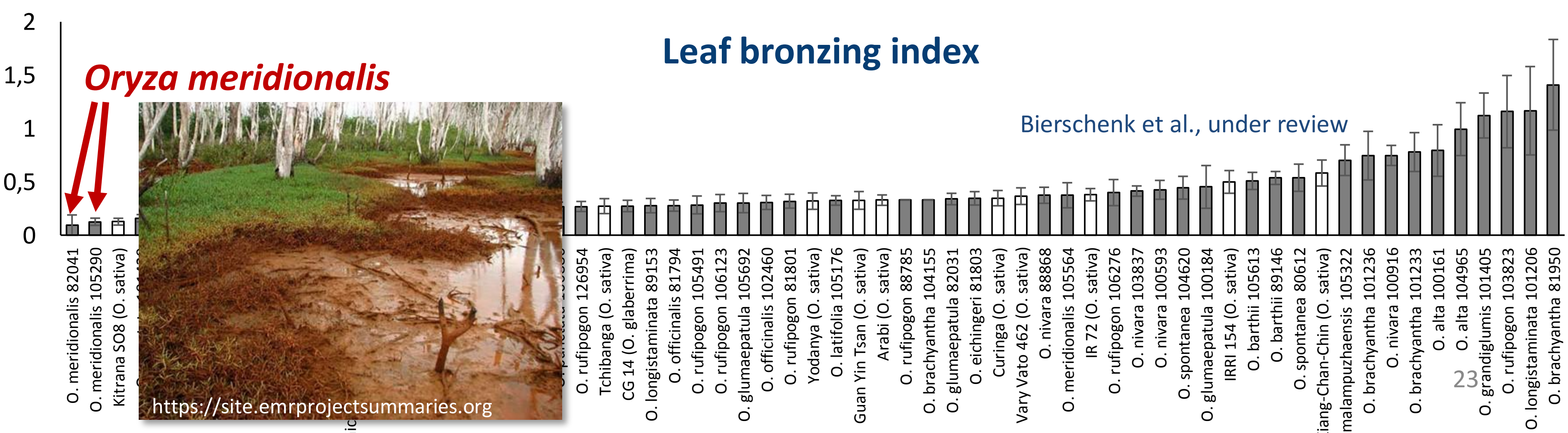
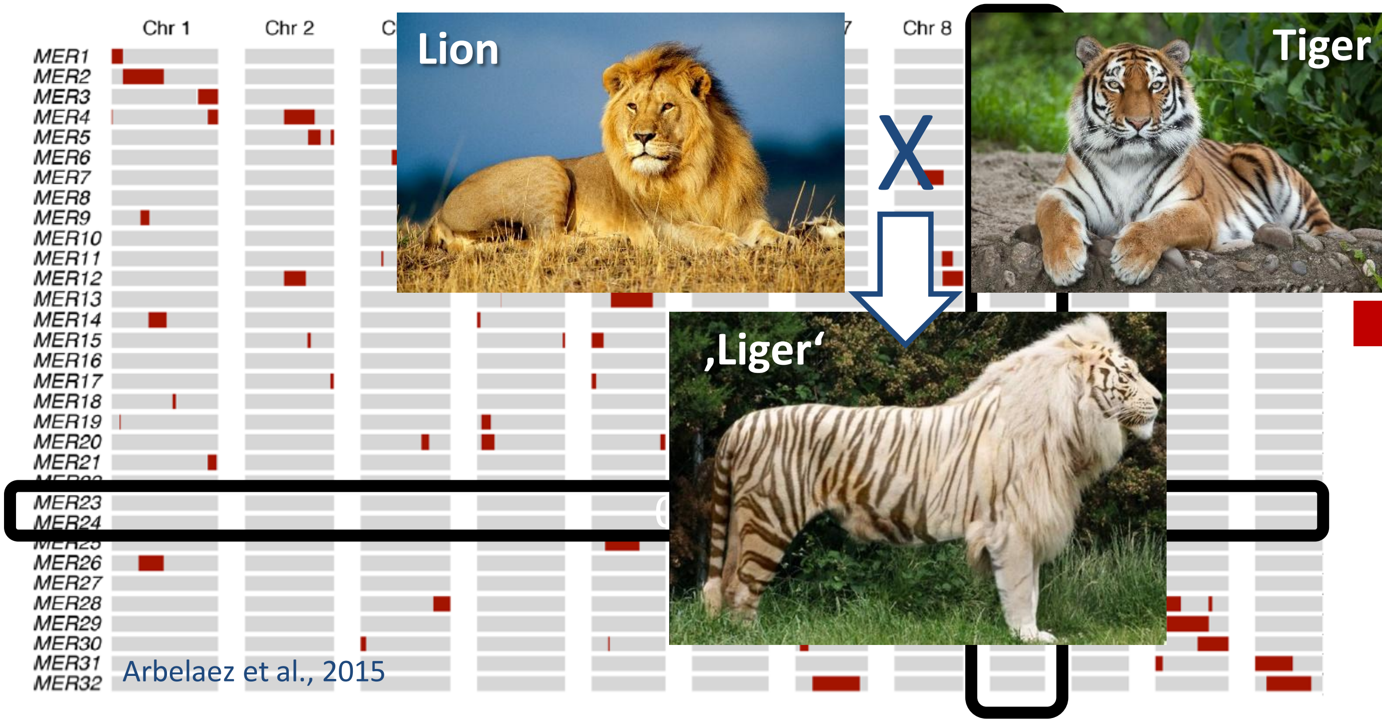
- Greenhouse experiment in soil
- Simulation of acute and chronic Fe stress
- 75 genotypes
  - 58 accessions of wild rice relatives (consisting of 20 species)
  - 16 *O. sativa* varieties
  - 1 *O. glaberrima* variety



Interspecific Crosses



Arbelaez et al., 2015

= *O. sativa* genome (variety Curinga from Brazil)

= chromosomal fragments from *O. meridionalis*

Hydroponic sceening experiments

- Control
- Fe stress treatment

Wairich et al., unpublished

Shoot Tolerance Mechanisms

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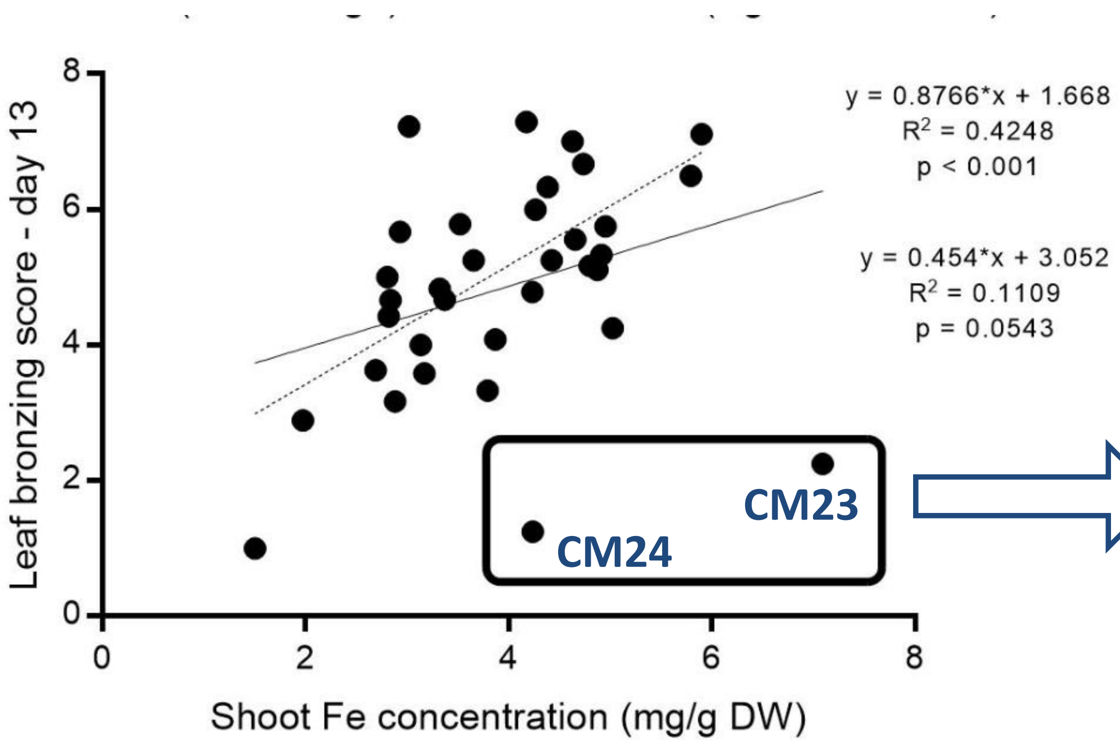
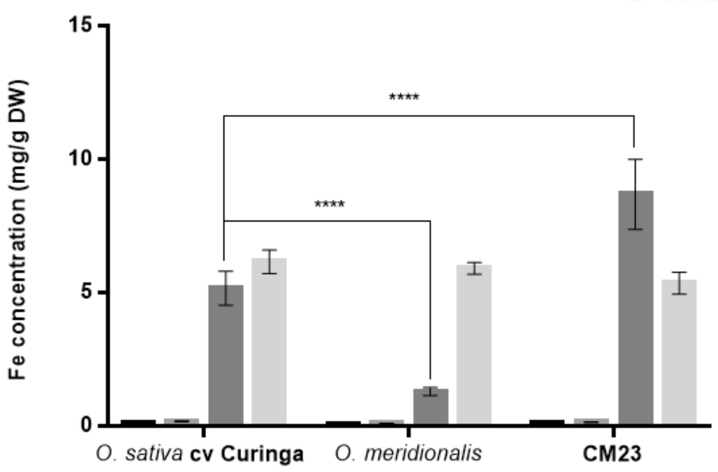


Wairich et al., unpublished

Few symptoms despite very high shoot Fe concentrations!

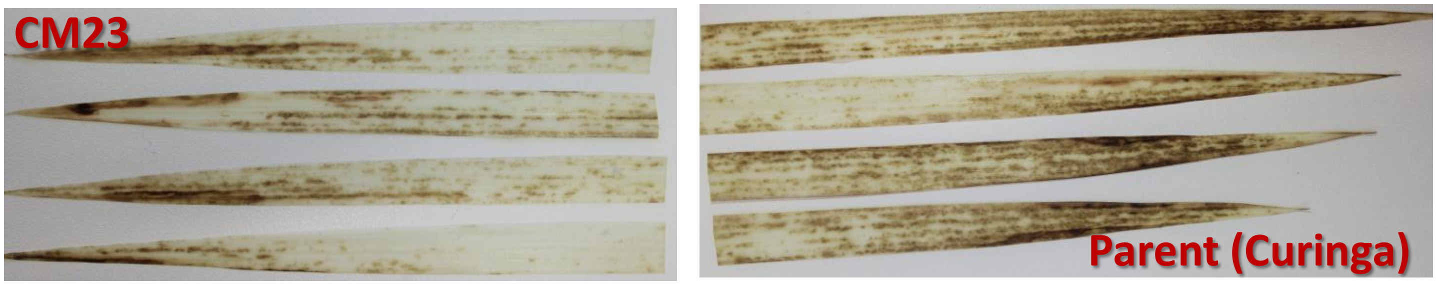


- Control stem
- Control leaf blade
- Fe stress stem
- Fe stress leaf blade

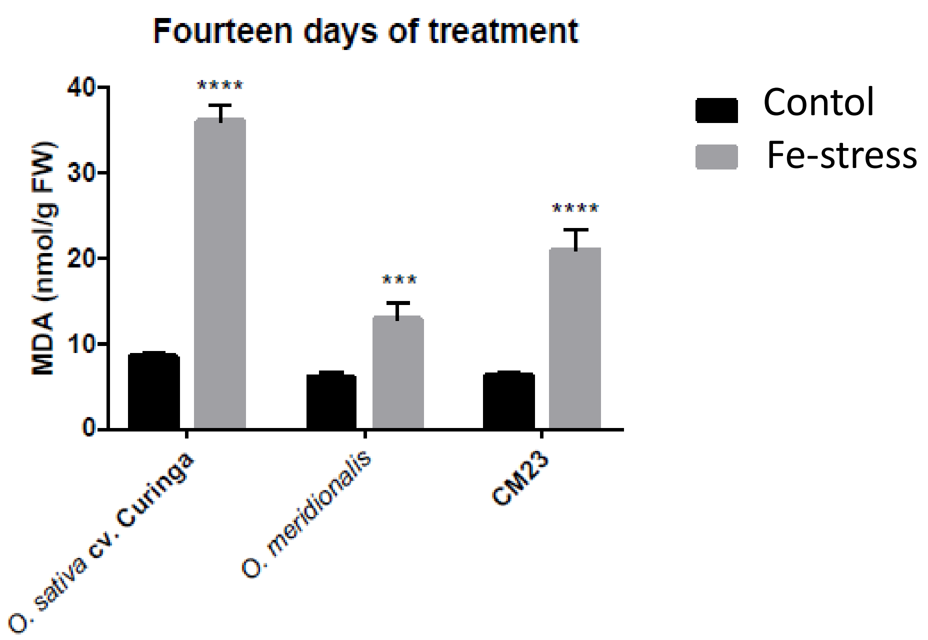
Tolerant CM23 retains excess Fe in the stem

Shoot Tolerance Reduces Oxidative Stress

H_2O_2 Staining



Lipid Peroxidationn



RNA-Sequencing in Interspecific Hybrids

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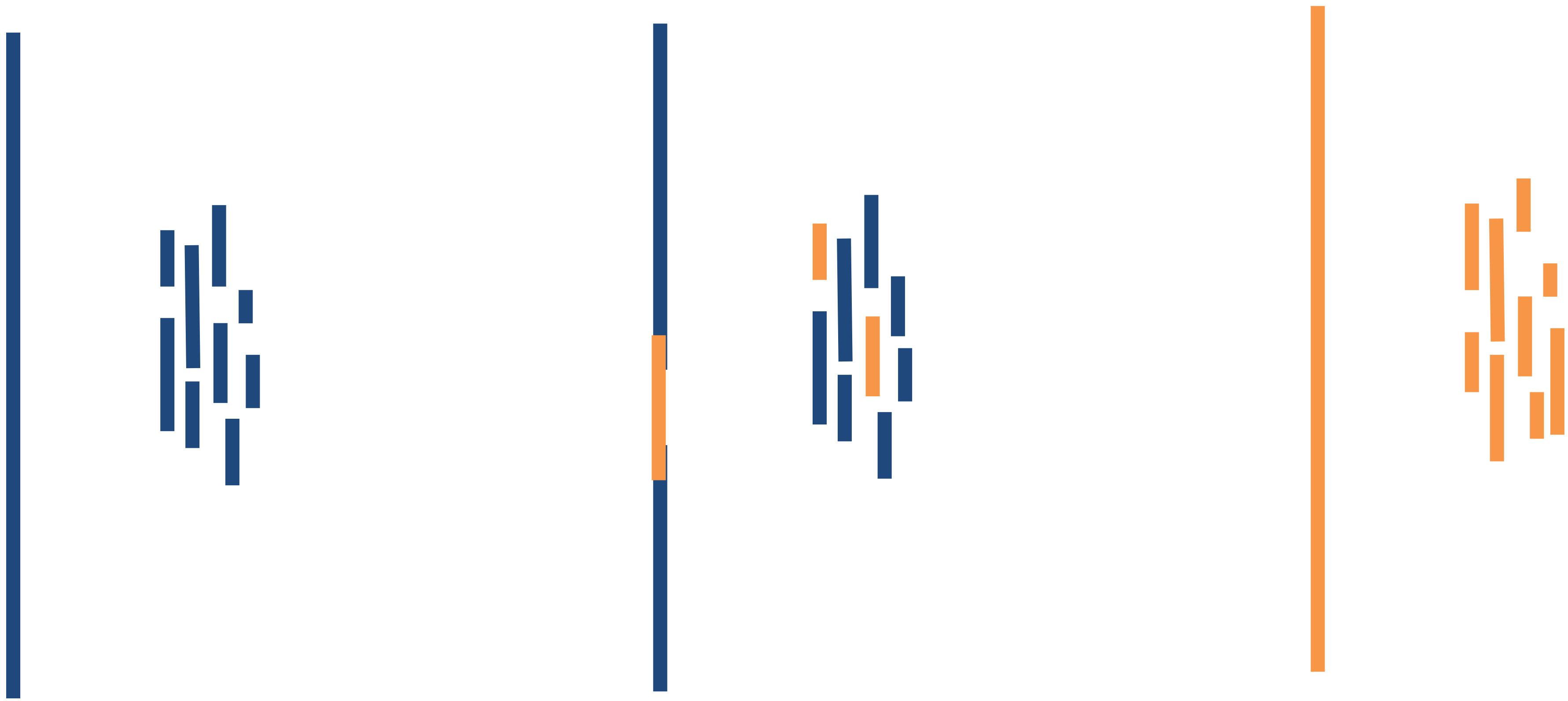

O. sativa reference genome

CM23 hybrid genome

O. meridionalis reference genome

⇨ Ongoing

Research Questions

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2. Can we use rice wild relatives for adaptation to Fe toxicity?

- Yes, some wild relatives show tolerance exceeding what we find in the primary gene pool of rice.
- Inclusion mechanisms in a *O. meridionalis* hybrid
- Genes and mechanisms remain to be characterized.

Research Questions

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3. Do tolerant varieties accumulate more Fe in their grains than intolerant ones?

Grain Fe Concentrations in Fe-toxic Conditions

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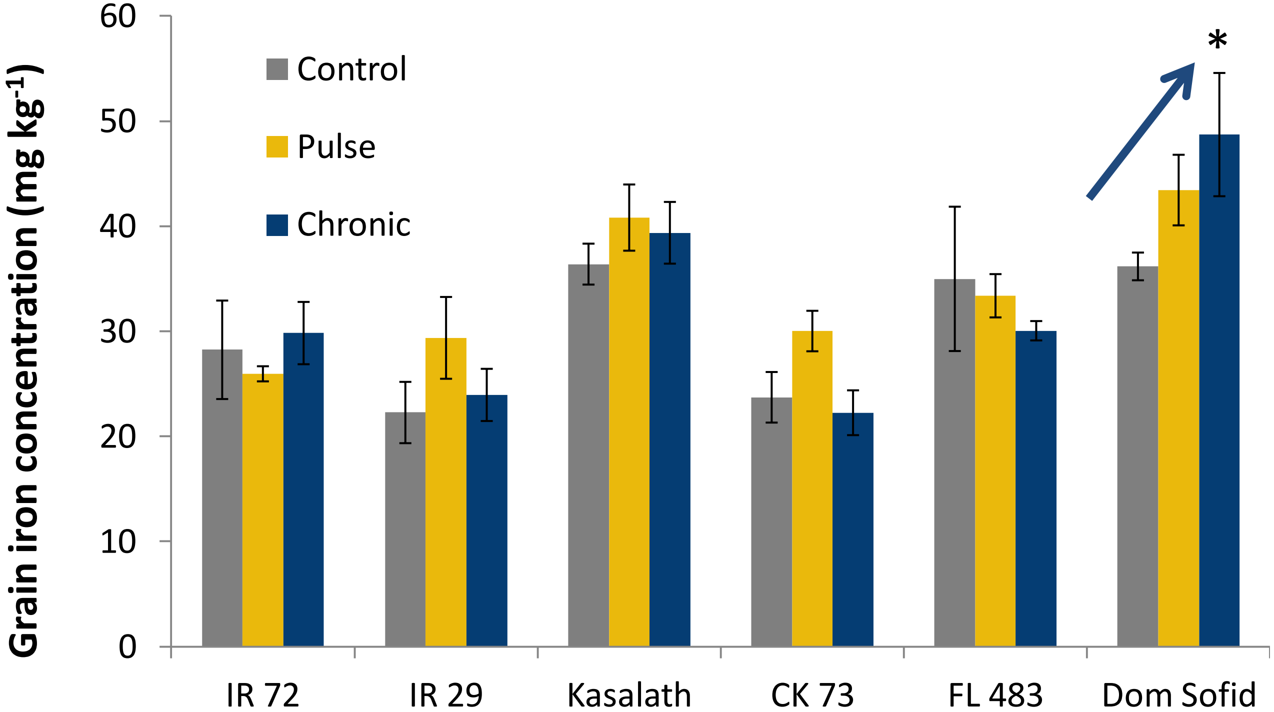


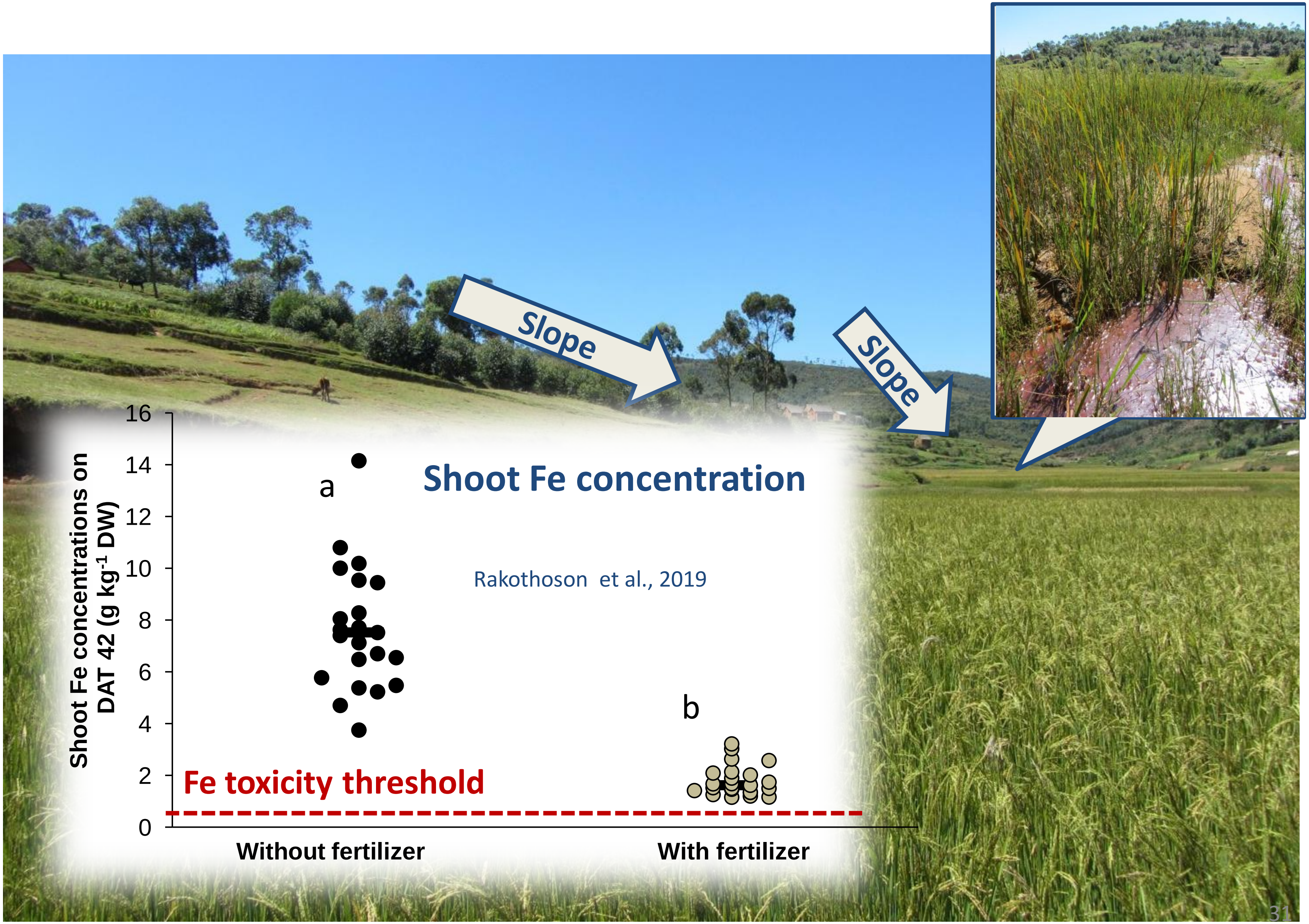
Frei et al., 2016

- Semi-artificial greenhouse plots
- Simulation of chronic and acute Fe stress

⇨ **In most varieties, grain Fe did not respond to excess Fe supply**

⇨ **Exception: Dom Sofid**

Field Experiments in Madasgacar



Grain Fe Concentrations of Field-Grown Rice

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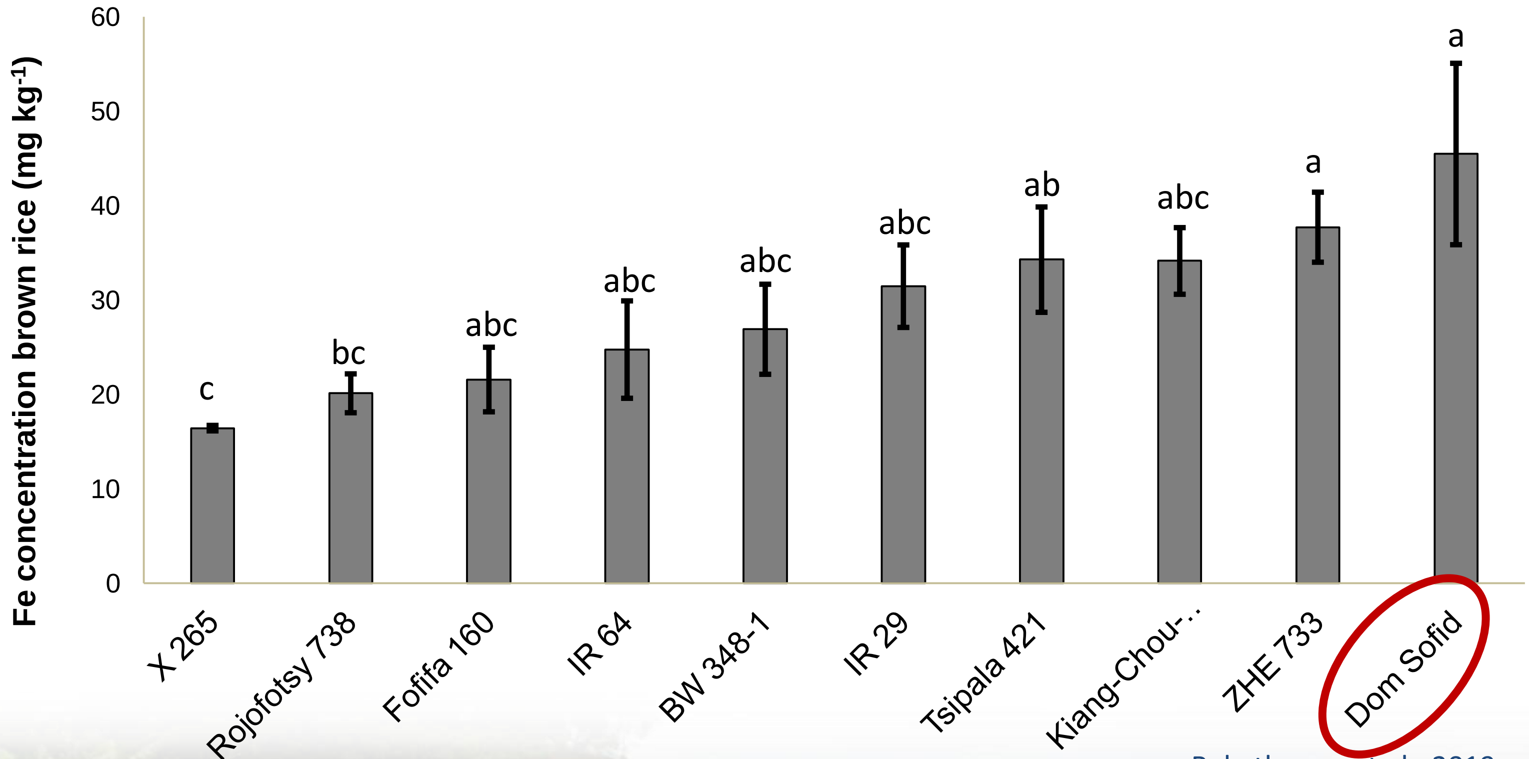

Rakothoson et al., 2019

Research Questions

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3. Do tolerant varieties accumulate more Fe in their grains than intolerant ones?

- So far only one variety identified that accumulates slightly more Fe in the grains when grown in Fe toxic conditions.

Conclusions

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- Root and shoot-based Fe-tolerance mechanisms identified in primary and secondary gene pool of rice

- Limited scope for increasing grain Fe using natural variation

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Thank you for your attention!

Research in Germany

DAAD

PhD scholarships

Alexander von Humboldt
Stiftung / Foundation

Postdoctoral fellowships

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