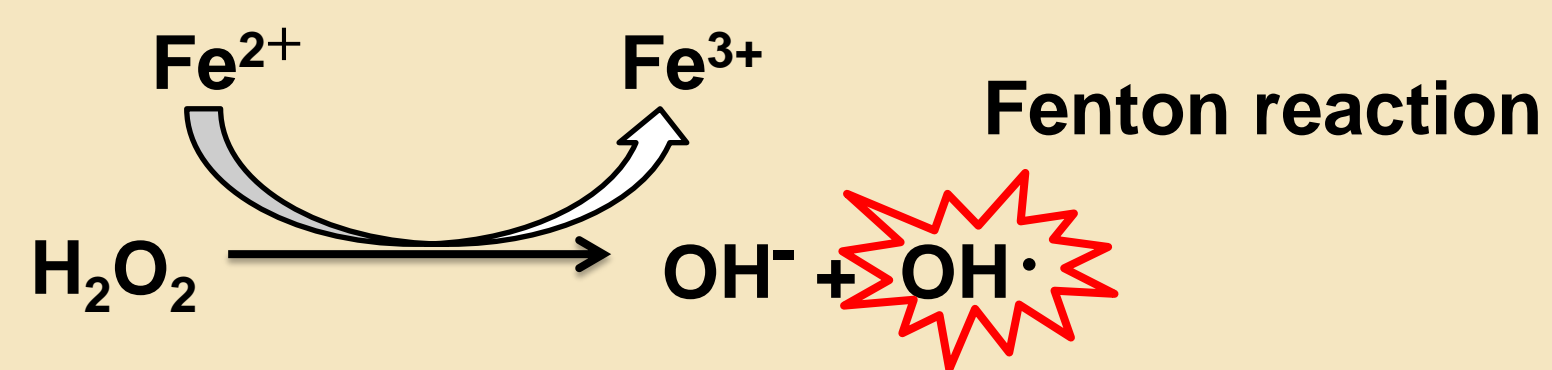


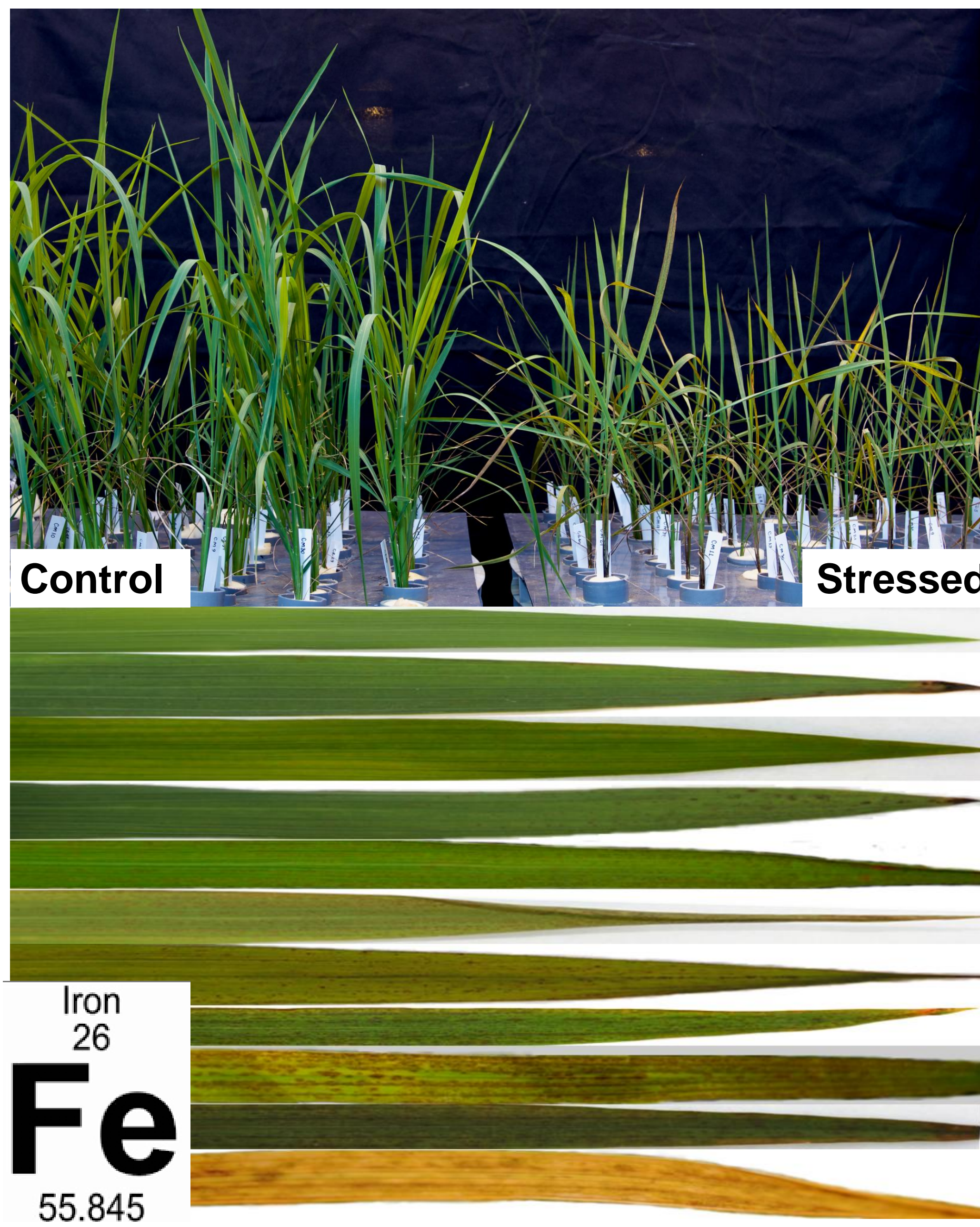
Confirmation and Functional Characterization of Potassium Ion Channel Gene for Iron Toxicity Tolerance in Rice

Background

Excessive uptake of Iron (Fe) leads to toxicity effects in lowland rice fields due to the high availability of ferrous (Fe^{2+}) in the soil solutions. Once the ferrous iron enters the plant cell it causes the generation of reactive oxygen species (ROS) via the so called Fenton reaction,

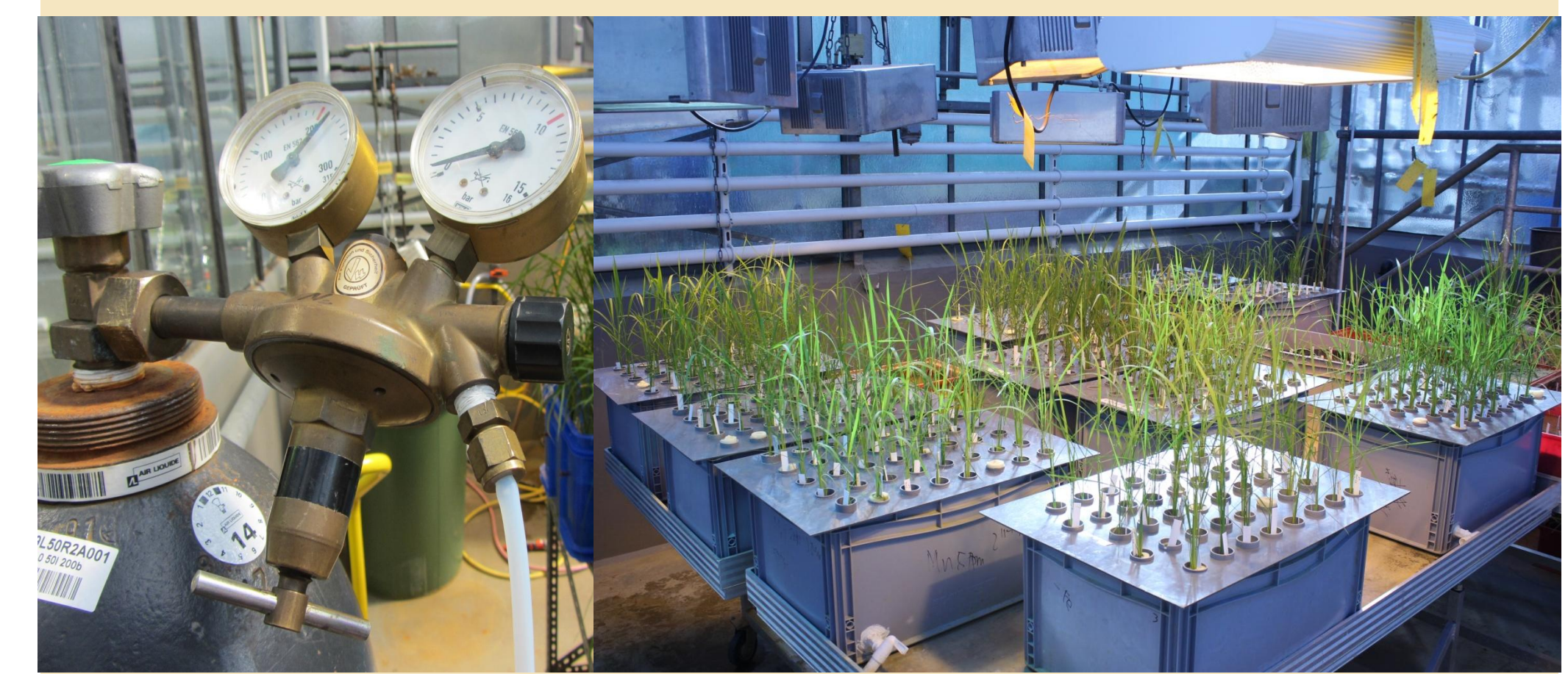


which can irreversibly damage cell structural components and impair physiological processes leading to yield reductions. In our previous study, quantitative trait loci related to the shoot iron concentration were investigated by genome-wide association study. One highly significant marker on chromosome 1 was located closely to a putative potassium ion channel gene (*OsAKT1*), which may be involved in Fe tolerance. The underlying physiological hypothesis is that iron might have antagonistic interactions with potassium or other ions. Furthermore potassium might be involved in limiting Fe translocation from the root to shoot.

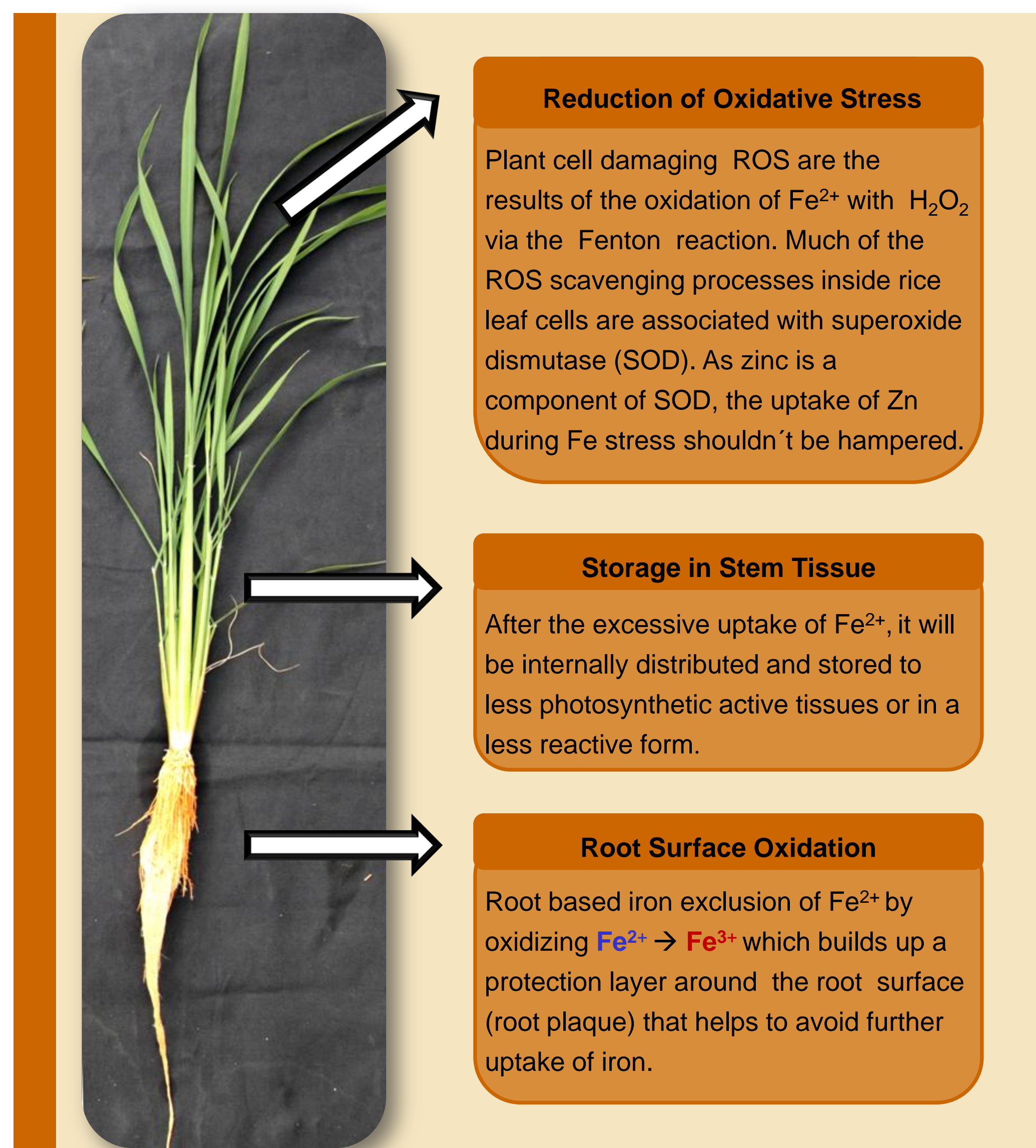


Materials and Methods

Rice plants (*Oryza sativa* L.) were grown for 5 weeks in a greenhouse in hydroponic containers filled with Yoshida nutrient solution. The solution was changed every 10d and the pH-value was adjusted to 5.5 every other day to ensure optimal growth conditions. In the 5th week the pre-cultured plants were exposed to iron stress of 1,000ppm Fe^{2+} for 5 days. The nutrient solution was automatically infused by a N_2 gas treatment of 15min every 2h to hamper the oxidation of Fe^{2+} to Fe^{3+} .



Tolerance Mechanisms



Reduction of Oxidative Stress

Plant cell damaging ROS are the results of the oxidation of Fe^{2+} with H_2O_2 via the Fenton reaction. Much of the ROS scavenging processes inside rice leaf cells are associated with superoxide dismutase (SOD). As zinc is a component of SOD, the uptake of Zn during Fe stress shouldn't be hampered.

Storage in Stem Tissue

After the excessive uptake of Fe^{2+} , it will be internally distributed and stored to less photosynthetic active tissues or in a less reactive form.

Root Surface Oxidation

Root based iron exclusion of Fe^{2+} by oxidizing $Fe^{2+} \rightarrow Fe^{3+}$ which builds up a protection layer around the root surface (root plaque) that helps to avoid further uptake of iron.

Conclusions

- ✓ *OsAKT1* was confirmed as Fe tolerance gene.
- ✓ Potassium, Zinc and Calcium showed antagonistic effect on excess Fe uptake.
- ✓ Fe stress significantly suppressed the expression of *OsAKT1* in the root but induced the expression in shoot tissue.
- ✓ Potassium status in rice plant regulates the transport of iron from root to shoot.
- ✓ Phenotypic data confirms higher susceptibility of *osakt1-1* and *osakt1-2* mutants compared to wild types in excess iron conditions.

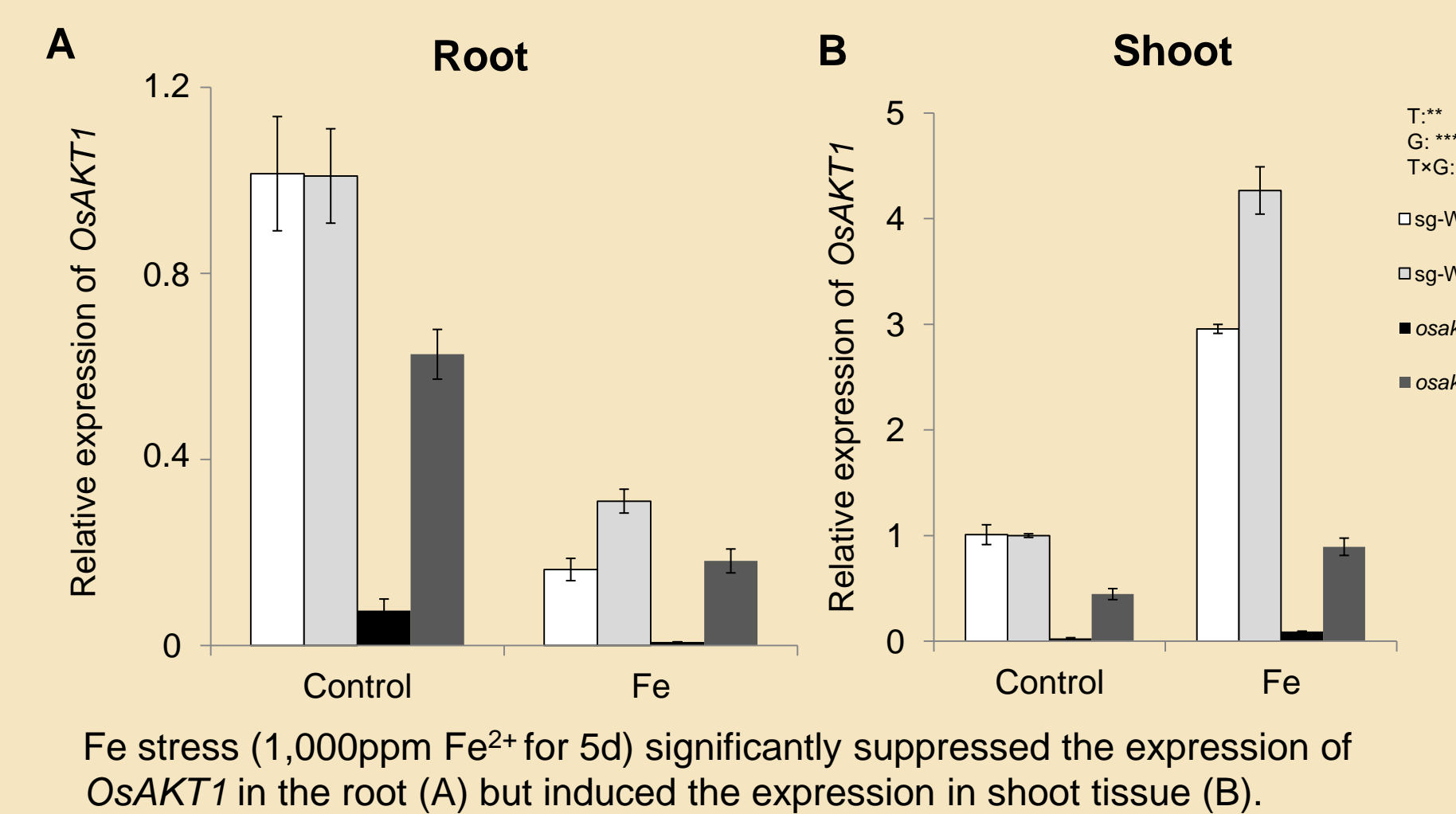
Candidate Gene

OsAKT1 Gene – Potassium Ion Channel Gene

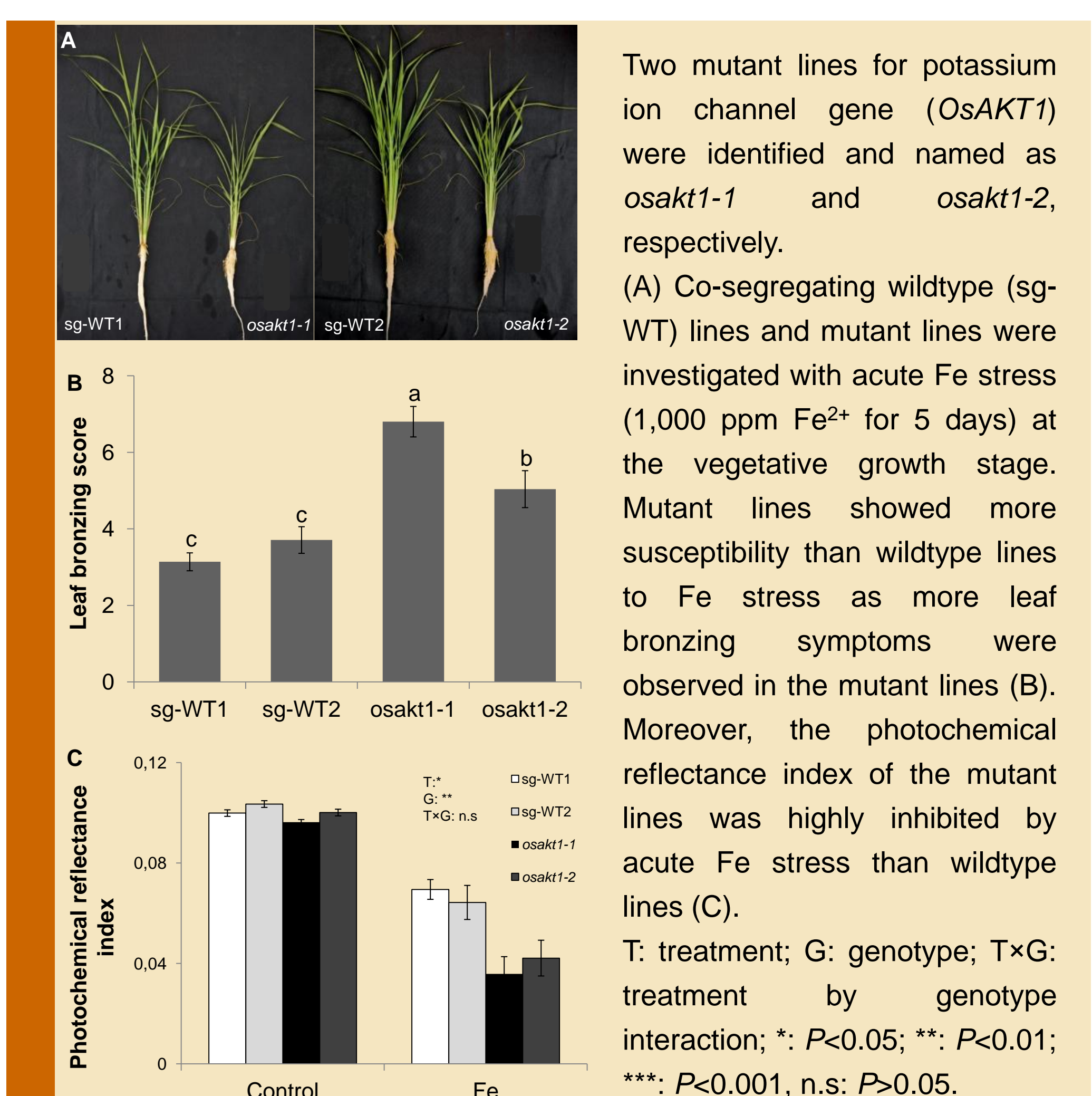
- LOC_Os01g45990.1
- 6.458kp long gene, located on chromosome 1
- Multi-pass membrane protein
- Expressed in roots and coleoptile

In order to proof, if K or other ions and Fe might have antagonistic interactions experiments were conducted with:

- WT (wildtype)
- sg-WT1, sg-WT2 (segregating wildtypes)
- *osakt1-1* and *osakt1-2* (homozygous knock-out mutants)



OsAKT1 – Phenotyping

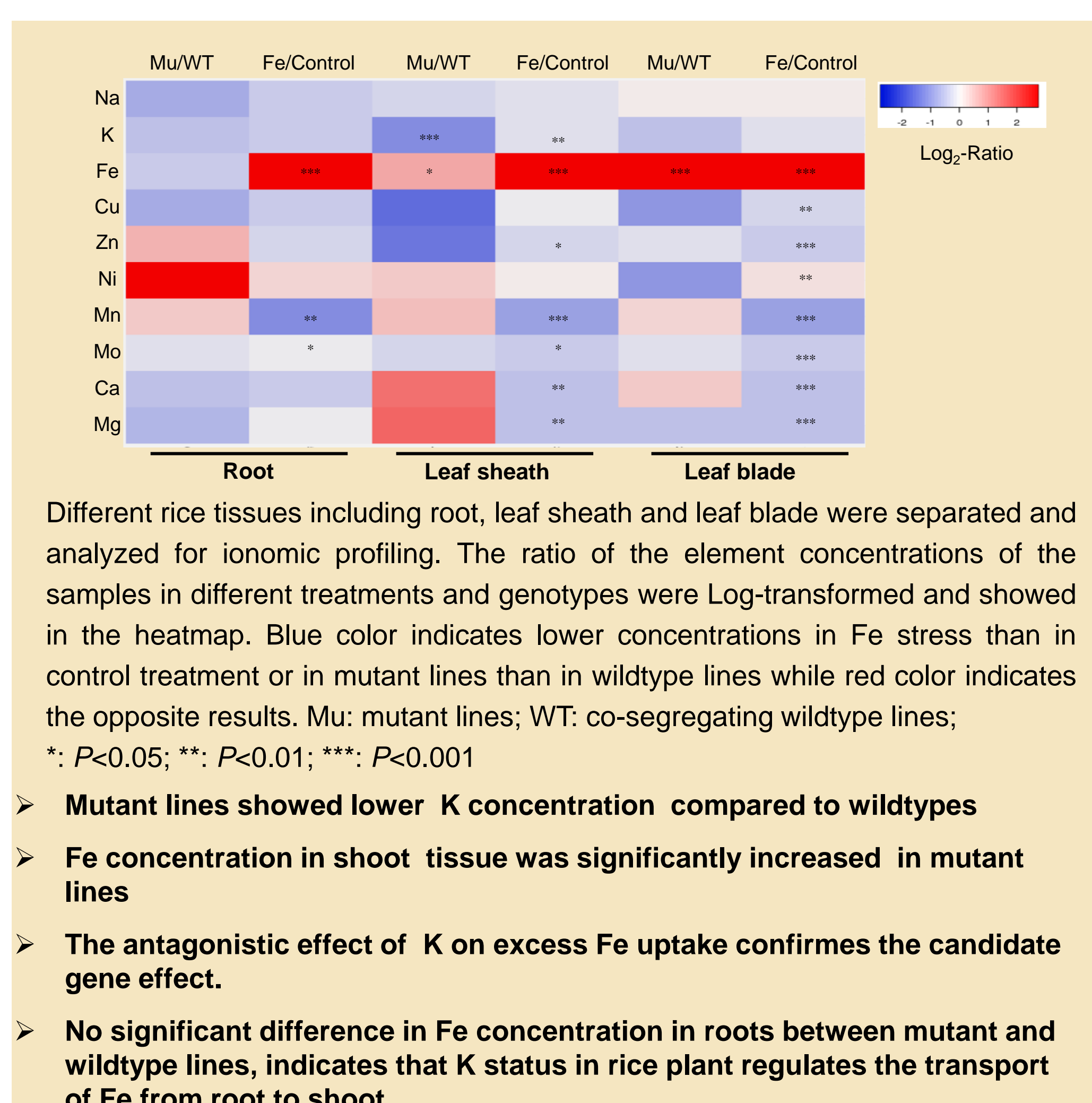


Two mutant lines for potassium ion channel gene (*OsAKT1*) were identified and named as *osakt1-1* and *osakt1-2*, respectively.

(A) Co-segregating wildtype (sg-WT) lines and mutant lines were investigated with acute Fe stress (1,000 ppm Fe^{2+} for 5 days) at the vegetative growth stage. Mutant lines showed more susceptibility than wildtype lines to Fe stress as more leaf bronzing symptoms were observed in the mutant lines (B). Moreover, the photochemical reflectance index of the mutant lines was highly inhibited by acute Fe stress than wildtype lines (C).

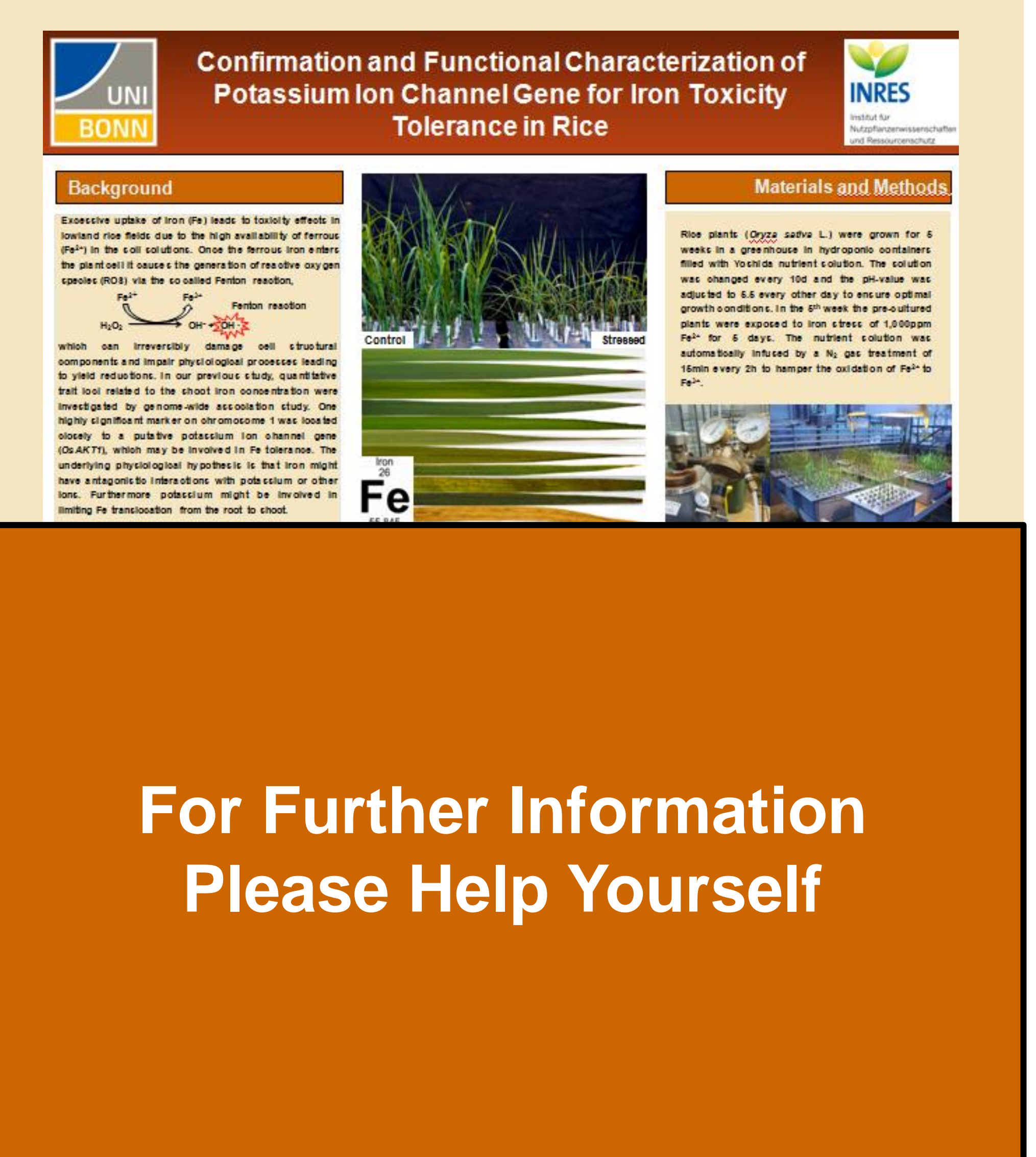
T: treatment; G: genotype; T×G: treatment by genotype interaction; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$, n.s.: $P > 0.05$.

OsAKT1 – Ionomics profiling



Different rice tissues including root, leaf sheath and leaf blade were separated and analyzed for ionomics profiling. The ratio of the element concentrations of the samples in different treatments and genotypes were Log₂-transformed and showed in the heatmap. Blue color indicates lower concentrations in Fe stress than in control treatment or in mutant lines than in wildtype lines while red color indicates the opposite results. Mu: mutant lines; WT: co-segregating wildtype lines; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

- Mutant lines showed lower K concentration compared to wildtypes
- Fe concentration in shoot tissue was significantly increased in mutant lines
- The antagonistic effect of K on excess Fe uptake confirms the candidate gene effect.
- No significant difference in Fe concentration in roots between mutant and wildtype lines, indicates that K status in rice plant regulates the transport of Fe from root to shoot.



For Further Information Please Help Yourself

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