Improved tolerance to iron toxicity in rice by knock-down of a citrate transporter FRDL1

Iron (Fe) toxicity is a nutrient disorder that specifically affects lowland rice, limiting its productivity in many regions such as Sub-Saharan Africa and Southeast Asia, typically resulting in yield losses of more than 10%. Despite its significance, our understanding of the genes and mechanisms to enhance Fe toxicity tolerance is limited, and breeding efforts have seen little success. Therefore, further investigation is needed to identify genes that can be used for tolerance breeding. In this study, the author focused on the fact that Fe within roots is transported to the shoot in the form of a citrate-Fe complex, and investigated the effects of mutating citrate transporters on Fe toxicity tolerance.

Wild-type plants, along with loss-of-function mutants for FRDL1 (a transporter that releases citrate in the xylem) and FRDL4 (a transporter that releases citrate to the rhizosphere) were evaluated in a hydroponic system with varying amounts and forms of Fe. Tolerance levels and the mechanisms were examined by measuring foliar symptoms and tissue Fe concentrations. The results showed that the *frdl1* mutant, but not the *frdl4* mutant, exhibited increased tolerance to Fe toxicity (Fig. 1). This suggests that xylem citrate plays a crucial role in determining Fe toxicity tolerance in rice plants, while citrate released in the rhizosphere is less important, at least in the hydroponic system. The *frdl1* mutant exhibited significantly lower Fe concentrations in leaf blades under excess ferrous iron (Fe^{2+}) conditions compared to wild-type plants, while root concentrations remained unaffected (Fig. 2). Contrary to the observation under excess Fe^{2+} conditions, reductions in leaf blade Fe concentrations were not observed under excess chelated Fe (Fe-EDTA) conditions. This suggests that the FRDL1 mutation alleviates Fe toxicity stress only when excess Fe is provided in the form of unchelated Fe^{2+}. To assess if the *frdl1* mutation could enhance tolerance in a sensitive variety, the *frdl1* mutant (Nipponbare background) was crossed with a sensitive *indica* variety, Ciherang, and the resulting F_2 population was evaluated under excess Fe^{2+} conditions. Individuals with the functional Ciherang-type FRDL1 exhibited severe symptoms with high leaf Fe concentrations, while those with the non-functional, mutated FRDL1 showed milder stress symptoms and significantly lower leaf Fe concentrations (Fig. 3).

These results suggest that a malfunctioning of FRDL1 leads to decreased xylem citrate concentrations, resulting in less efficient root-to-shoot Fe translocation (Fig. 4). This indicates that FRDL1 is a promising target to reduce leaf Fe concentration under Fe toxicity conditions. Further research is necessary to understand the factors that regulate the expression of *FRDL1* in roots and apply this knowledge to practical breeding efforts.

Author: Ueda, Y. [JIRCAS]

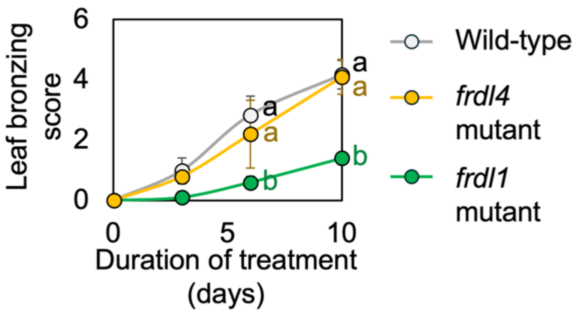

Fig. 1. Formation of leaf symptoms in different genotypes

The degree of leaf symptoms under excess ferrous iron (Fe^{2+}) conditions was quantified by "leaf bronzing score." Higher values indicate more severe symptom formation. Different alphabets indicate significant differences (p<0.05).

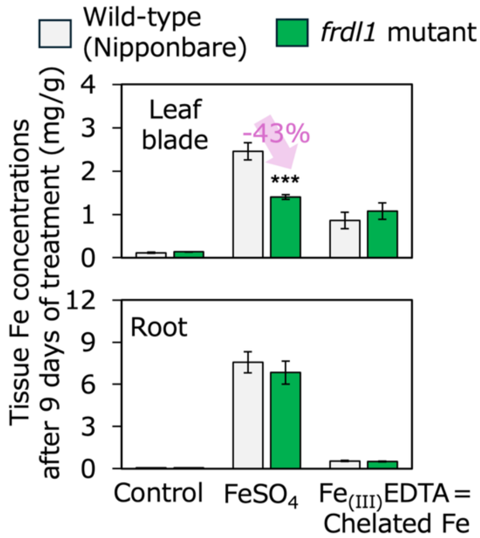

Fig. 2. Tissue Fe concentrations under different treatments

Fe concentrations in leaf blades and roots were measured under the control, excess ferrous iron ($FeSO_4$), and excess chelated iron (Fe-EDTA) conditions. Asterisks (***) indicate that the concentration was significantly different between the wild-type and frdl1 mutant at p<0.001 level.

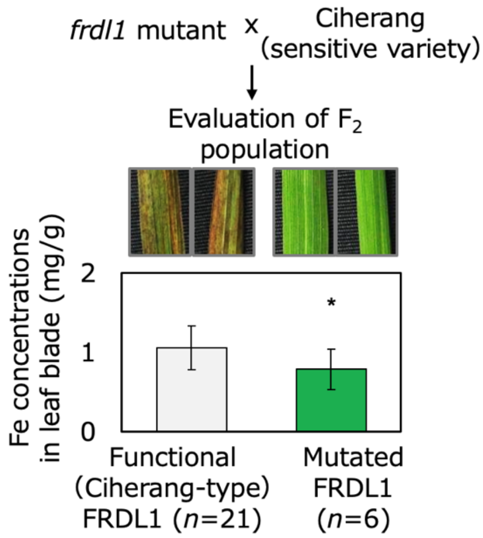

Fig. 3. Effects of FRDL1 mutation in the sensitive Ciherang background

Leaf traits and leaf blade Fe concentrations were evaluated in the F_2 population. Leaves of representative plants are shown. The asterisk (*) indicates that Fe concentrations are significantly different between plants with the functional FRDL1 and mutated FRDL1 at p<0.05 level.

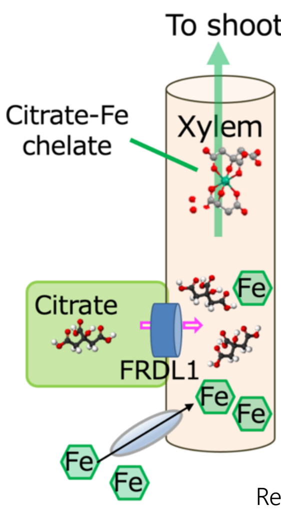

Fig. 4. Possible mechanism for the mitigation effect of Fe toxicity stress in the FRDL1 knock-down line

FRDL1 plays a crucial role for exuding citrate to the xylem. Xylem contains Fe absorbed by plants, and chelation by citrate leads to more efficient translocation of Fe to the shoot. FRDL1 knock-down decreases xylem citrate concentrations, and the concomitant decrease in chelated Fe leads to reduced Fe translocation to the shoot. Chelated Fe (Fe-EDTA) can be transported to the shoot without citrate, and the effects of FRDL1 mutation is not evident.

Reference: Ueda, Y. (2025) *Plant Biology*, https://doi.org/10.1111/plb.70107.