Iron accumulation in the endosperm: where is the bottleneck?

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Abstract
Rice contains low level of iron in the starchy endosperm, which contributes to a widespread iron deficiency in some population groups of rice-based diet countries. Although understanding iron loading in the grain is a cardinal step to define better biofortification strategies, this had received little attention so far. We use interdisciplinary approaches to closely explore how rice seeds load iron. Iron content in the seeds during grain development of different varieties is quantified using ICP-OES, whereas the quantity of phytosiderophores is determined by HPLC, which allows a functional analysis of iron transport mechanisms. We correlated these results with the expression levels of selected genes involved in iron transport. Candidate genes will be proposed for engineering a new generation of plants to elucidate iron loading in the seeds. Our research gives a new insight on iron loading in the seeds and we discuss the new data in the optic to develop new strategies for iron biofortification of rice.

Media summary
Understanding the iron fluxes and spatio-temporal accumulation of iron in the rice grain. Enhancing iron content in the endosperm.

Keywords
Micronutrient, nutrition, biofortification, grain

Introduction
In developing countries, some population groups suffer from severe micronutrient malnutrition due to a lack of sufficient micronutrients such as vitamin A, zinc, and iron in the diet. These groups are characterized by high intakes of staple food crops such as maize, wheat and rice, but low consumption of foods rich in bioavailable micronutrients such as fruits, vegetables, and animal and fish products. Iron deficiency is the most prevalent nutritional deficiency worldwide, with about 3.5 billion iron deficient people in developing countries based on levels of blood hemoglobin, indicator of anemia. Certain population groups such as women of reproductive age and preschool children are more vulnerable to iron deficiency. Aside direct micronutrients interventions such as food fortification and supplementation, a biofortification strategy is based on the development of micronutrient-dense staple crops. Previous studies have consistently shown that iron content in the rice grain can be enhanced by expressing a plant ferritin protein in the endosperm leading to a two-fold increase of iron content in the polished grain. However, such enhancement is not sufficient to alleviate iron deficiency. One impediment to iron content enhancement in the grain is the lack of knowledge about iron distribution and the kinetics of accumulation in the developing grain. For example, it is clear that most of iron is mainly located in the aleurone layer and in the embryo and it is known that vascular bundles are very important for grain filling, especially at early stage of the grain development. However, it is unclear how exchanges between the embryo and the developing endosperm occur. Our

objective is thus to unravel unknown mechanisms of iron loading in the rice grain that may lead to new strategies for iron biofortification of rice.

Method

Seeds of one japonica cultivar (Tsukinohikari) and two indica cultivars (IR64 and IR68144) were harvested harvested at four different developmental stages, i.e. early milky, late milky, dough and mature stage. Seeds were dehulled and maternal layers were detached. If necessary, endosperm and embryo were separated. These material were used for analytical and gene expression analysis.

Seeds were dried at 65C for 5 days and stored a 4C until use. All seed material was prepared using ceramic tools to avoid iron contamination. About 200-300 mg rice seeds and 30-40 mg of rice embryo were weighed on a precision balance and digested in Teflon tubes using 1 ml HNO3 and 1 ml H2O2 ICP grade in a microwave (MARSS EXPRESS, CEM, USA) for 40 min at 220C and 1200W. Mineral content was measured by ICP-AES (SPS1200VR ICP-AES, Seiko, Japan). The iron content in the endosperm was calculated as difference between the content in the full grain and in the embryo.

Results

Previous studies about endosperm development showed that compartmentalization and cellularization of the rice endosperm is completed 5 days after fertilization (DAF). Cell division in the endosperm and embryo ends at 10DAF. The kinetic of starch accumulation in the rice grain indicates that proplastids appear 4 DAF in the endosperm cells. In addition, the central part of the endosperm is more rapidly filled with substance reserves than the outer layers (visible 10DAF). Several strategies have been envisaged to enhance iron content in the grain and can be summarized in two main approaches 1) expressing of the ferritin protein in the endosperm and 2) enhancing iron uptake and iron fluxes in the grain by enhancing transporters activity through vascular system elements. We and others have shown that the specific expression of plant ferritin in the endosperm can indeed enhance the iron content in the polished grain (data not shown). So far, the promoters driving the expression of the ferritin are mostly active 5 days after fertilization and we are currently evaluating different promoters to enhance the production of ferritin at earlier stages of the grain development with the assumption that iron fluxes occur within the developing grain at early stages. In order to validate this approach and to propose alternative strategies, we are currently investigating iron fluxes. The iron fluxes between the different systems and the possible strategies are summarized in figure 1. Each system can be described by its volume (size), concentration of different elements, and flux directions and rate are key parameters to understand the dynamics of exchange between two systems. The intensity of the exchanges or fluxes is directly controlled by the boundary type and the duration of fluxes between two systems. Each strategy can thus be described by the number of systems and boundaries controlling the iron fluxes (figure 1). For example, the strategy based on the expression of the ferritin in the endosperm is based on one system only and a strategy combining ferritin and enhancement of iron loading in the grain is a two systems/one boundary approach. With the available data, it is however difficult to assess the likelihood of success of a particular strategy. We focus this particular study to iron fluxes occurring within the panicle to better assess the one system or two system strategies (figure 1).

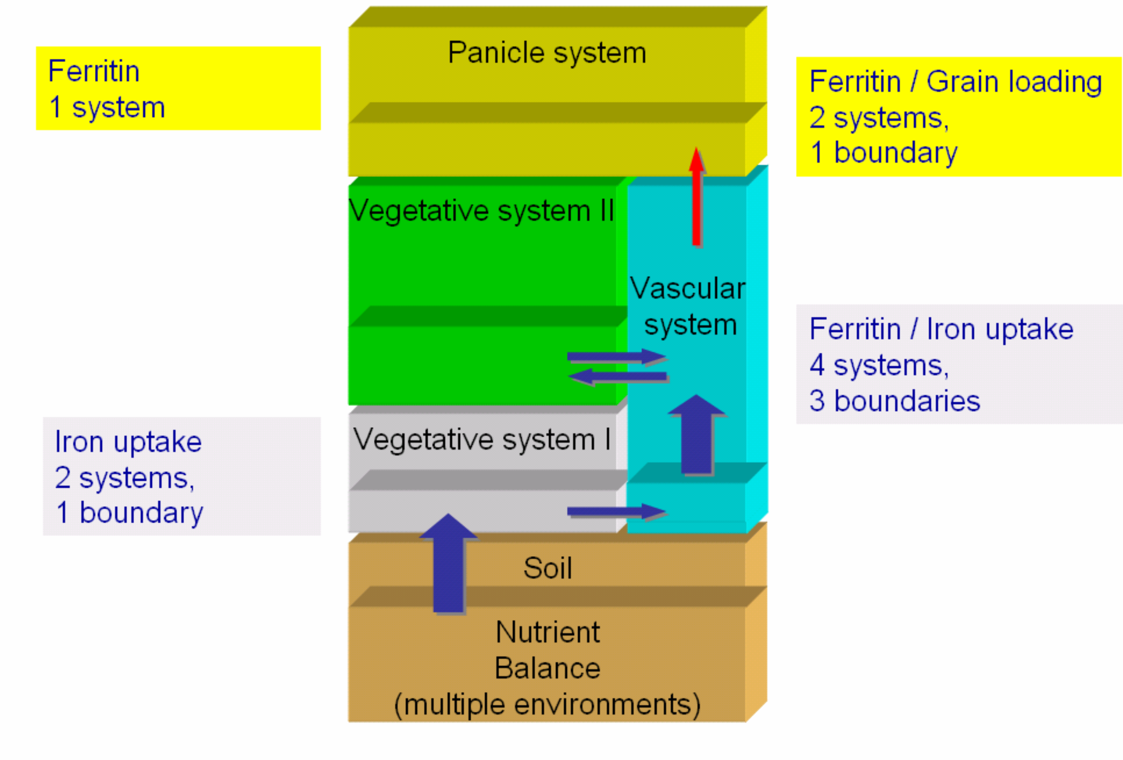

Figure 1 Iron fluxes between systems and possible biofortification strategies

Preliminary results in mature seeds of Tsukiinohikari highlighted that the iron concentration (Fe) in the embryo is about 10-fold higher than in the endosperm. The predominant form of Fe is DMA but it is possible that the predominant form of Fe is different at an early stage of grain development. Based on expression data of iron transporters such as OsYSL2 and OsYSL15, it is hypothesized that the embryo can load iron from the maternal tissue very efficiently and germinating embryo can load iron from the endosperm (NK Nishizawa, personal communication).

To achieve a successful biofortification strategy, we have chosen to generate several populations of elite indica IR64 and IR68144 containing enhanced iron content in the endosperm. It has been repeatedly shown that the native level of iron in IR68144 is significantly higher than IR64. These two varieties exhibit distinctive panicle architecture and grain shape and the relations of these parameters with iron content are under investigation. IR68144 grains are clearly smaller in all dimensions than IR64 (figure 2). We have successfully generated a large number of transgenic events containing different combinations of endosperm specific::plant ferritin (soybean and rice) in both varieties. Our overall approach is based on developing the biofortification trait as a native trait. Toward that specific goal, we have determined the content of several micronutrients in these two varieties that will be presented at the meeting.

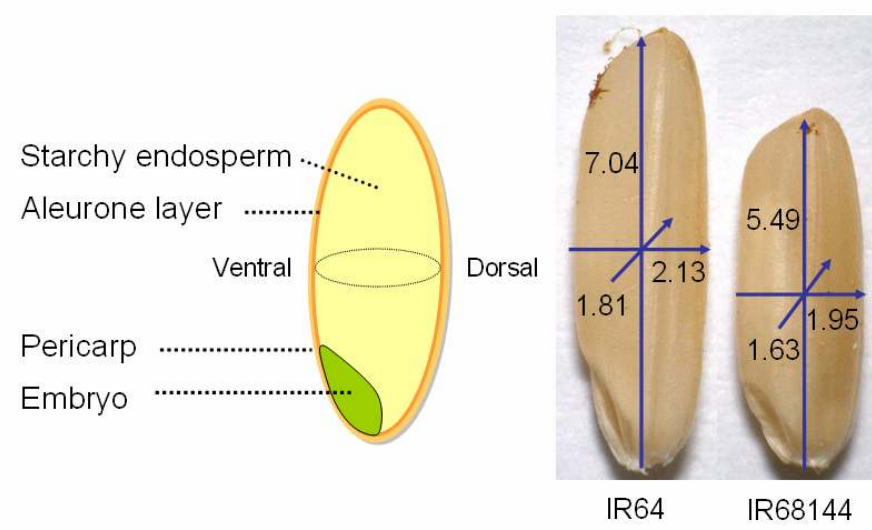

Figure 2 Grain parameters of IR64 and IR68144

Conclusion

Biofortification of staple food crops is a global initiative to alleviate micronutrient deficient in some population groups in developing countries and to contribute to poverty reduction. Several strategies have been envisaged and for some crops such as rice, the use of genetically modified varieties is a strategy that may allow achieving micronutrient level requirements. We propose to develop such strategy as a native trait. By integrating different biochemical and genomics-based methodologies, a better understanding of the iron distribution within the panicle and the grain and the kinetics of iron loading in the developing grain can greatly facilitate the assessment of the different strategies toward the identification of the most likely successful biofortification trait development.