EFFECTS OF DIFFERENT IRON SUPPLIES ON WHOLE-PLANT MINERAL PARTITIONING AND REMOBILIZATION TO THE RICE PANICLES

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INTRODUCTION

Minimal information exists on whole-plant dynamics of mineral flow through rice plants or on the source tissues responsible for mineral export to developing seeds. Understanding these phenomena in a model plant could help the development of nutritionally enhanced crop cultivars. Despite the increasing number of studies about the physiology and regulation of uptake from the rhizosphere for several minerals, the lack of knowledge about how minerals are moved into or out of vascular tissues, translocated to vegetative tissues and loaded into seeds is one of the barriers to biofortification of seeds (KRAMER et al. 2007).

Initial seed biofortification efforts in rice showed higher accumulation of minerals in leaves than in grains of the transgenic plants, which indicates that translocation of minerals to seeds is tightly regulated, and that simply increasing net mineral uptake into the plant will probably not result in seeds with higher mineral concentrations. It is already known that minerals may be remobilized from vegetative sources, although a major portion of minerals in seeds are likely supplied through continuous uptake and translocation during reproductive growth to developing seeds (HIMELBLAU AND AMASINO, 2001).

In this work, we assess growth dynamics of the whole plant (panicles, non-flag leaves, flag leaves, stems/sheaths and roots) over the reproductive development of the model plant *Oryza sativa* and describe the accumulation of ten mineral nutrients (Fe, Zn, Cu, Mn, Mo, Ni, Ca, Mg, K and S) in these organs over time. Additionally, we studied the net loss of mineral contents to investigate the potential of vegetative tissues as sources of stored minerals to be remobilized to panicles. To address the question of whether different Fe supplies could influence mineral partitioning and movement of minerals to panicles, we compare these parameters in plants treated with low (5 μM), normal (20 μM) and high (200 μM) Fe concentrations.

MATERIAL AND METHODS

Plant materials and growth conditions

Rice seeds from the fast-growing cultivar Kitaake were germinated in petri dishes for 8 d before being transferred to hydroponic solution. Plants were grown in a controlled environment chamber with 16-h, 20°C day and 8-h, 15°C night at the USDA-ARS Children's Nutrition Research Center, Houston, TX. The standard solution for hydroponically grown plants contained 1 mM $Ca(NO_3)_2$, 3 mM KNO_3, 0.5 mM $MgSO_4$, 0.75 mM K_2SO_4, 0.5 mM KH_2PO_4, 25 µM $CaCl_2$, 25 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.5 µM H_2MoO_4, 0.1 µM $NiSO_4$, 0.1 mM K_2SiO_3, and 20 µM Fe(III)-HEDTA. All nutrients were buffered with 2 mM MES (2,4-morpholino-ethane sulfonic acid), pH 5.5 and growth solutions were replaced

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every 3 days. For treatments involving different Fe concentrations, Fe(III)-HEDTA concentration was adjusted to 5, 20 or 200 μM. O delineamento experimental utilizado foi inteiramente casualizado.

Elemental analysis by ICP

All tissues were harvested and dried in a 60°C oven for 48 h. Dried tissues were predigested overnight in borosilicate glass tubes with 4 ml of redistilled 98.8 % HNO_3. One milliliter of concentrated trace metal grade $HClO_4$ was added to the predigested tissues and heated at 100°C for 1 h, 150°C for 1 h, 180°C for one hour and then at 210°C to dryness (1-2 h). Digests were resuspended in 15 ml of redistilled 2% HNO_3. Concentrations of Fe, Zn, Cu, Mn, Mo, Ni, Ca, Mg, K and S were determined by inductively coupled plasma-optical emission spectroscopy (CIROS ICP Model FCE12, Germany). Mineral content was determined by multiplying each sample's concentration by dry weight. For estimation of net mineral content loss from non-flag leaves, flag leaves and stems/sheaths, the final mineral content of each organ was subtracted from the time point with the highest mineral content. For minerals that had a decrease in content, the net loss was compared with final total panicle mineral content to determine the contribution of remobilized minerals to panicle mineral content.

Partition quotient calculation

To evaluate the partitioning of minerals within a rice plant during its reproductive development, changes in each tissue's content were normalized to changes in each tissue's weight, relative to the whole plant. The DW of each organ was calculated as a percentage of total plant weight at each time point, and mineral content of each organ was calculated as a percentage of total plant mineral content at each time point. Using these values, the normalized partitioning of that mineral within the plant was calculated by dividing each organ's percentage mineral content by its percentage DW, and multiplying by 100, which we refer to as the partition quotient (PQ).

Statistical analyses

When appropriate, data were subjected to analyses of variance (ANOVA) and means were compared by the Tukey HSD (Honestly Significant Differences) ($P \leq 0.05$). The Levene's test (for homogeneity of variance) was used prior to ANOVA.

RESULTS AND DISCUSSION

Effects of low and high Fe supplies on mineral dynamics

According to Figure 1, low Fe supply (5 μM) promoted higher accumulation of Zn, Cu and Ni in roots; Mn, Ca, Mg and K in leaves and Zn in stems/sheaths. On the other hand, it promoted smaller accumulation of Fe, Mn and Ca in roots and Zn and Ni in leaves. Enhancement of Zn uptake under low Fe supply could explain its higher accumulation found in roots. Several Fe transporters are able to transport also Zn and Mn (LÓPEZ-MILLÁN et al. 2004). Induction of Fe transporters under low Fe concentration could result in increased Zn uptake and translocation to stems/sheaths and increased Mn uptake and quick translocation to leaves (RAMANI AND KANNAN, 1987). Possibly, uptake of Mn and Cu may increase in rice plants under low Fe supply, because the presence of phytosiderophores in the rhizosphere may increase the availability of these ions both in the rhizosphere itself and in the apoplast (ZHANG et al. 1991). Increases in Mn, Mg, and K accumulation were associated with Fe deficiency in pea, suggesting that plasma membrane reductases may have a regulatory function in root ion-uptake processes via their influence on the oxidation-reduction status of the membrane (WELCH et al. 1993). Eckhardt et al. (2001) proposed that the Fe transporters IRT1 and IRT2 from tomato could also transport Ni^{2+}.

High Fe supply (200 μM) promoted higher accumulation of Fe in roots and Zn in leaves. On the other hand, it promoted a smaller accumulation of Fe in leaves and stems/sheaths and Zn, Cu and K in roots (Figure 1). As expected, high Fe supply resulted in higher Fe accumulation in roots. Most part of this Fe seems to be not translocated to shoots, since smaller Fe PQ values were detected in leaves and stems/sheaths (Figure 1). In accordance with our results, Silveira et al. (2007) detected higher Zn concentration in shoots of plants under Fe-excess. Also in accordance to our results, smaller K (MEHRABAN et al. 2008) and Cu (SILVEIRA et al. 2007) absorption rates under Fe-excess have already been reported in rice.

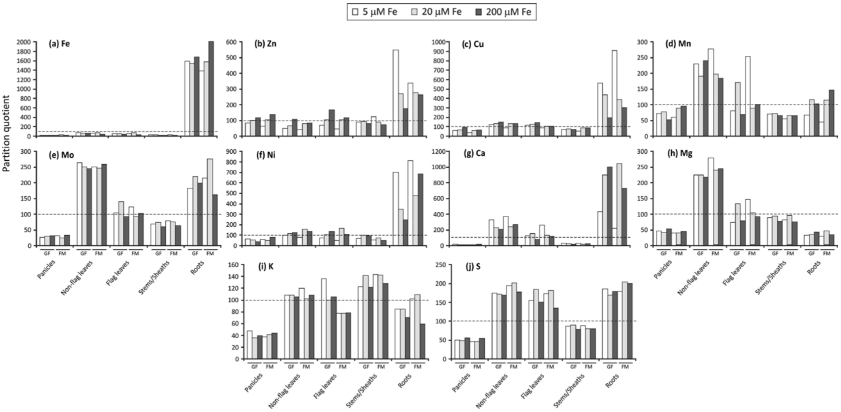

Figure 1. Partition quotients (PQ) in panicles, non-flag leaves, flag leaves, stems/sheaths and roots during grain filling (GF) and full maturity (FM) stages of rice plants cultivated with 5, 20 or 200 μM of Fe(III)-HEDTA. Dashed horizontal line represents PQ of 100 (dry weight increase of the organ is responsible for its increased mineral content).

Mineral remobilization versus continued supply from roots

Plants watered with a high Fe concentration (200 μM) showed only K and S remobilization from flag leaves and Ni from stems/sheaths (Figure 2c). In this case, vegetative tissues should have been able to store Fe quantities above the structural minimum, which would have provided excess Fe for remobilization. However, with the abundant Fe supply at the root level, continued uptake during seed fill may have reduced or precluded the need for remobilization to serve as a source of Fe for seeds. On the other hand, plants watered with low Fe concentration (5 μM) showed the highest Fe remobilization (Figure 2a), probably due to reduced uptake during seed fill. Under limited Fe supply, stems/sheaths seem to be the major Fe source for remobilization. All the minerals analyzed showed remobilization when plants were watered with an optimal Fe concentration (20 μM), mostly from flag leaves, but also from stems/sheaths (Figure 2b). These results suggest that mineral remobilization from vegetative tissues can occur in rice plants; however, it is not absolutely required for seeds to acquire minerals.

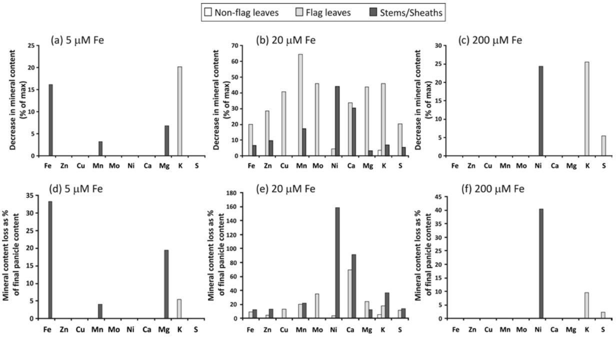

Figure 2. Percentage decrease in non-flag leaves, flag leaves and stems/sheaths mineral contents from rice (a) and

potential contribution of remobilized mineral to panicle mineral contents at the final time point (b).

According to our results, mineral remobilization from vegetative tissues in rice is greatly dependent of plant Fe nutrition, since non-optimal Fe supplies prevent the remobilization of several minerals. Also, a major proportion of mineral content in panicles probably comes from non-storage sources, that is, continued root uptake and translocation during the seed fill period. We did observe mineral remobilization mostly from flag leaves but also from stems/sheaths under 20 μM Fe, and at least a portion of these minerals was likely incorporated into panicles and seeds. In the unlikely event that 100% of the content of each mineral lost from vegetative tissues went to panicles, this could account for, at most, 20% of panicle Fe, Zn, Cu, Mn, Mg, K and S (Figure 2e). However, at least for Zn, it is already known that most of the mineral transported out of the leaves (either the flag leaf or the lowest senescent leaf) is translocated to other vegetative organs instead of being partitioned to the panicles, and even less to the grains (JIANG et al. 2007). We can also discard 100% Ni and Ca remobilization to panicles, since it would be responsible for more than 100% of the final panicle content (Figure 2e). However, a significant part of this remobilized Ni should be transported to the seeds, since it is already known that Ni rapidly re-translocates from leaves to young tissues in the phloem. Indeed, up to 70% of Ni in the shoots was transported to the seeds of soybean (TIFFIN, 1971).

The results shown in this work indicate that flag leaf is the organ with higher percentage of mineral content loss. However, considering that flag leaves have extremely low mineral content, the maximum possible contribution to panicle mineral content, assuming that the total net loss of each mineral was translocated to panicles before the final collection point, is in general higher from stems/sheaths than from flag leaves.

CONCLUSION

This study suggests that mineral accumulation throughout the reproductive development of rice organs can be affected by different Fe supplies. With respect to the translocation of minerals to panicles, remobilization from vegetative organs can occur in rice plants (and non-optimal Fe supplies can affect mineral remobilization), but apparently it is not absolutely required for seeds to acquire minerals. In this way, continued uptake and translocation of minerals during seed fill are probably more important than remobilization of previously stored shoot minerals.

REFERENCES

- Eckhardt U, Mas Marques A, Buckhout TJ (2001) Two iron-regulated cation transporters from tomato complement metal uptake-deficient yeast mutants. Plant Molecular Biology 45: 437-448.
- Himelblau E, Amasino RM (2001) Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. Journal of Plant Physiology 158: 1317-1323.
- Jiang W, Struik PC, Lingna J, van Keulen H, Ming Z, Stomph TJ (2007) Uptake and distribution of root-applied or foliar-applied ^{65}Zn after flowering in aerobic rice. Annals of Applied Biology 150: 383-391.
- Krämer U, Talke IN, Hanikenne M (2007) Transition metal transport. FEBS Letters 581: 2263-2272.
- López-Millán A-F, Ellis DR, Grusak MA (2004) Identification and characterization of several new members of the ZIP family of metal transporters in *Medicago truncatula*. Plant Mol Biol 54: 583-596.
- Mehraban P, Zadeh AA, Sadeghipour HR (2008) Iron toxicity in rice (*Oryza sativa* L.) under different potassium nutrition. Asian Journal of Plant Sciences 7: 251-259.
- Ramani S, Kannan S (1987) Manganese absorption and transport in rice. Physiol Plant 33: 133-137.
- Silveira VC, Oliveira AP, Sperotto RA, Espindola LS, Amaral L, Dias JF, Cunha JB, Fett JP (2007) Influence of iron on mineral status of two rice (*Oryza sativa* L.) cultivars. Braz J Plant Physiol 19:127-139.
- Tiffin LO (1971) Translocation of nickel in xylem exudates of plants. Plant Physiology 48: 273-277.
- Welch RM, Norvell WA, Schaefer SC, Shaff JE, Kochian LV (1993) Induction of iron(III) and copper(II) reduction in pea (*Pisum sativum* L.) roots by Fe and Cu status: Does the root cell plasmalemma Fe(III)-chelate reductase perform a general role in regulating cation uptake? Planta 190: 555-561.
- Zhang FS, Römheld V, Marschner H (1991) Diurnal rhythm of release of phytosiderophores and uptake rate of zinc in iron-deficient wheat. Soil Science and Plant Nutrition 37: 671-678.