WHOLE-PLANT MINERAL PARTITIONING DURING THE REPRODUCTIVE DEVELOPMENT OF RICE (*Oryza sativa* L.)

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INTRODUCTION

Plants are the primary source of nutrients for human nutrition. Staple seed crops such as rice supply the majority of daily dietary nutrients for billions of people. However, the rice grain has low density of mineral nutrients, and for those whose diets are high in staple foods, micronutrient malnutrition is widespread. To keep up with the population growth and to improve the nutrition and health of rice consumers, development of high-quality rice varieties becomes increasingly important (DUAN AND SUN, 2005).

Despite the increasing number of studies about the physiology and regulation of uptake of several minerals from the rhizosphere, 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).

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*. We also describe the concentrations and contents of ten mineral nutrients (Fe, Zn, Cu, Mn, Mo, Ni, Ca, Mg, K and S) in these organs over time.

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 every 3 days.

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.

Partition quotient calculation

To evaluate the partitioning of minerals within a rice plant during its reproductive

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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. Pearson's correlation analyses were carried out using two significance levels ($P \leq 0.05$ and 0.01). All the statistical analyzes were performed using the SPSS Base 19.0 for Windows (SPSS Inc., USA).

RESULTS AND DISCUSSION

Growth and mineral dynamics

According to our analysis, three different patterns of mineral accumulation during the reproductive development of rice could be detected. First, Fe, Zn, Cu and Ni are preferentially accumulated in the roots (Figures 1a, b, c and f). Silveira et al. (2007) also detected lower Fe levels in shoots than in roots of different rice cultivars. A large part of the Fe in plants is in the apoplast, particularly the root apoplast. Most of this apoplastic pool is in the basal roots and older parts of the root system (RÖMHELD AND NIKOLIC, 2007). We found Fe PQ values of about 100 in non-flag and flag leaves (Figure 1a). It is already known that a significant proportion of Fe is also localized within the chloroplast of rapidly growing leaves (MARSCHNER, 1995). According to Römheld and Nikolic (2007), concentrations of Fe in seeds are lower than in the vegetative organs, corroborating our extremely low Fe PQ values in panicles (Figure 1a). Zn PQ values decrease in roots throughout the reproductive development of rice, along with non-flag leaves (Figure 1b). On the other hand, Zn PQ values increase in stems/sheaths. Simmons et al. (2003) reported the temporary accumulation of Zn in stems. In panicles, Zn PQ values are higher during panicle exertion (PE) than in grain filling (GF) and full maturity (FM) stages. Indeed, it has been shown that high Zn accumulation during early seed development is possibly related to protein synthesis. There are several reports showing that protein synthesis in seeds is particularly high during early seed development (MARTRE et al. 2003) and Zn is the most critical micronutrient affecting protein synthesis in plants (OBATA et al. 1999). Cu has limited transport in plants; therefore, the highest concentrations are often in root tissues (CHAIGNON et al. 2002). Analysis of 16 different forage species revealed that root tissues accumulated the highest Cu concentrations (28.8 mg kg^{-1}), followed by leaves (15.5 mg kg^{-1}) and stems (8.4 mg kg^{-1}) (PEDERSON et al. 2002). Similar pattern was found in our work (Figure 1c). Nickel distribution in plants depends on their developmental stage. Thus, most Ni accumulation in the roots of *Avena sativa* was registered at the tillering and booting stages (in accordance with our result) (ANDREEVA et al. 2000). Also similar to our results, Dwivedi et al. (2007) found that most of the Ni was confined to roots in all the three tested rice cultivars.

The second pattern of mineral accumulation involves Mn and Mg, which are accumulated in leaves (Figure 1d and h). Mn moves easily from the root to the shoot in the xylem-sap transpirational stream. In contrast, re-translocation within the phloem is complex, with leaf Mn being immobile, but root and stem Mn being able to be re-mobilized (LONERAGAN, 1988), which could explain high Mn PQ values in leaves and low PQ values in roots and stems/sheaths. In cucumber, Mg concentrations were seven times higher in the shoots than in the roots. Yet, Mg accumulation in the younger leaves of cucumber is higher after flowering and fruiting (BENGTSSON AND JENSEN, 1983), which is similar to our flag leaves Mg PQ values (Figure 1h).

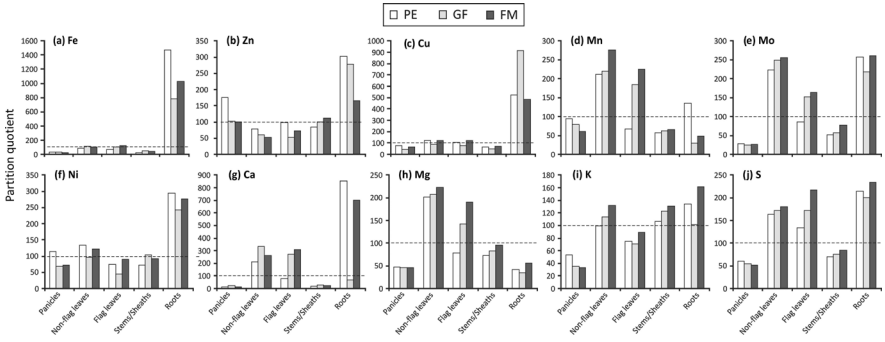

Figure 1. Partition quotients (PQ) in panicles, non-flag leaves, flag leaves, stems/sheaths and roots during panicle exertion (PE), grain filling (GF) and full maturity (FM) stages of rice plants cultivated under control condition (20 μM Fe(III)-HEDTA). Dashed horizontal line represents PQ of 100 (dry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

The third pattern of mineral accumulation involves Mo, Ca, and S, which are accumulated in roots and leaves (Figure 1e, g and j); and K, which is accumulated in roots, leaves and stems/sheaths (Figure 1i). Generally, the concentration of Mo in crop species is higher in leaves than in the stems (GUPTA AND LIPSETT, 1981). Surprisingly, Ca PQ value decrease in roots during GF stage (Figure 1g). Most of this Ca content was probably transported to leaves, which accumulate more Ca during GF than PE stage. We found higher S concentrations in roots than in leaves, but in general, photosynthetically active leaves show the highest S concentrations of all plant organs (HANEKLAUS et al. 2007). It is already known that K is taken up from the soil solution at high rates and is quickly distributed in plant tissues and cell organelles, owing to the low- and high-affinity channels. Potassium ions cycle via xylem from roots to upper plant parts and via phloem from leaves to roots. The direction depends on the physiological demand. However, for optimum grain filling, a high K concentration in the leaves is required for the translocation of assimilates to the grains and for protein synthesis in these grains (MENGEL et al. 1981).

Pearson correlation analysis indicated that Fe-Mn and K-S were positive correlated in every analyzed organ. Fe-Ni, Cu-Mg and Mn-Ni were positive correlated in four of the five organs, showing a negative correlation in roots (Figure 2). Zeng et al. (2005) showed a significant positive correlation between Fe and Mn content in brown rice. Parida et al. (2003) observed that Fe contents in plants of *Trigonella* increased with the increase in the Ni concentration applied. Zeng et al. (2005) also showed a significant positive correlation between Cu and Mg in brown rice. Fe and Zn positive correlation, found in three of the five analyzed tissues (Figure 2), have already been reported in rice grains (SPEROTTO et al. 2009).

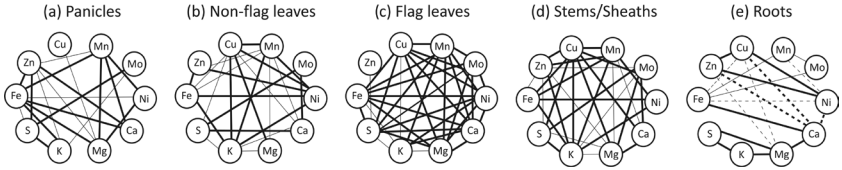

Figure 2. Pearson's correlation analyses of ten mineral concentrations in (a) panicles, (b) non-flag leaves, (c) flag leaves, (d) stems/sheaths and (e) roots through the reproductive development of rice plants cultivated with 20 μM of Fe(III)-HEDTA. Solid lines represent a significant positive correlation and dashed lines represent a significant negative correlation. Thinner lines indicate significance at the 0.05 level and thicker lines indicate significance at the 0.01 level.

CONCLUSION

While Fe, Zn, Cu and Ni are preferentially accumulated in roots, Mn and Mg are accumulated in leaves; Mo, Ca, and S in roots and leaves; and K in roots, leaves and stems/sheaths. Correlation analyzes indicate that fluctuations in Fe-Mn, K-S, Fe-Ni, Cu-Mg and Mn-Ni concentrations throughout the reproductive development of rice were positively correlated in at least four of the five organs.

REFERENCES

- Andreeva IV, Govorina VV, Yagodin BA, Dosimova OT (2000) Dynamics of nickel accumulation and distribution in oat plants. Agrokhimiya 4: 68-71.
- Bengtsson B, Jensen P (1983) Uptake and distribution of calcium, magnesium and potassium in cucumber of different age. Physiologia Plantarum 57: 428-434.
- Chaignon V, DiMalta D, Hinsinger P (2002) Fe-deficiency increases Cu acquisition by wheat cropped in a Cu-contaminated vineyard soil. New Phytologist 154: 121-130.
- Duan M, Sun SSM (2005) Profiling the expression of genes controlling rice grain quality. Plant Molecular Biology 59: 165-178.
- Dwivedi S, Tripathi RD, Srivastava S, Mishra S, Shukla MK, Tiwari KK, Singh R, Rai UN (2007) Growth performance and biochemical responses of three rice (*Oryza sativa* L.) cultivars grown in fly-ash amended soil. Chemosphere 67: 140-151.
- Gupta UC, Lipsett J (1981) Molybdenum in soils, plants, and animals. Advances in Agronomy 34: 73-115.
- Haneklaus S, Bloem E, Schung E, de Kok LJ, Stulen I (2007) *Sulfur*. In: Barker AV, Pilbeam DJ, eds. Handbook of Plant Nutrition. Boca Raton, FL.: CRC Press, Taylor & Francis Group, pp. 183-238.
- Krämer U, Talke IN, Hanikenne M (2007) Transition metal transport. FEBS Letters 581: 2263-2272.
- Loneragan JF (1988) *Distribution and movement of manganese in plants*. In: Graham RD, Hannam RJ, Uren NC, eds. Manganese in Soils and Plants. Dordrecht: Kluwer Academic Publishers, pp. 113-121.
- Marschner H (1995) Mineral Nutrition of Higher Plants, 2nd edn. Academic Press, London.
- Martre P, Porter JR, Jamieson PD, Triböi E (2003) Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulation of nitrogen remobilization for wheat. Plant Physiology 133: 1959-1967.
- Mengel K, Secer M, Koch K (1981) Potassium effect on protein formation and amino acid turnover in developing wheat grain. Agronomy Journal 73: 74-78.
- Obata H, Kawamura S, Senoo K, Tanaka A (1999) Changes in the level of protein and activity of Cu/Zn-superoxide dismutase in zinc deficient rice plant, *Oryza sativa* L. Soil Science and Plant Nutrition 45: 891-896.
- Parida BK, Chhibba IM, Nayyar VK (2003) Influence of nickel-contaminated soils on fenugreek (*Trigonella corniculata* L.) growth and mineral composition. Scientia Horticulturae 98: 113-119.
- Pederson GA, Brink GE, Fairbrother TE (2002) Nutrient uptake in plant parts of sixteen forages fertilized with poultry litter: Nitrogen, phosphorus, potassium, copper, and zinc. Agronomy Journal 94: 895-904.
- Römheld V, Nikolic M (2007) *Iron*. In: Barker AV, Pilbeam DJ, eds. Handbook of Plant Nutrition. Boca Raton, FL.: CRC Press, Taylor & Francis Group, pp. 329-350.
- 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. Brazilian Journal of Plant Physiology 19:127-139.
- Simmons RW, Pongsakul P, Chaney RL, Saiyasitpanich D, Klinphoklap S, Nobuntou W (2003) The relative exclusion of zinc and iron from rice grain in relation to rice grain cadmium as compared to soybean: Implications for human health. Plant and Soil 257: 163-170.
- Sperotto RA, Ricachenevsky FK, Duarte GL, Boff T, Lopes KL, Sperb ER, Grusak MA, Fett JP (2009) Identification of up-regulated genes in flag leaves during rice grain filling and characterization of *OsNAC5*, a new ABA-dependent transcription factor. Planta 230: 985-1002.
- Zeng YW, Shen SQ, Wang LX, Liu JF, Pu XY, Du J, Qiu M (2005) Correlation of plant morphological and grain quality traits with mineral element contents in Yunnan rice. Rice Science 12: 101-106.