

# Dynamic Change of Zinc Distribution in Rice Plants

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## INTRODUCTION

Zinc (Zn) is an essential nutrient that plays important roles in numerous physiological processes in plants, serving as a cofactor for many enzymes and as the key structural motifs in transcriptional regulatory proteins. The uptake and translocation of Zn in plants is essential for plant growth, and since plants are the primary source of food for humans, the nutritional value of plants is of central importance to human health. Over 50% of the world's populations are suffered from micronutrient deficiency. Zn deficiencies are especially prevalent nutritional disorder in humans.

A deficiency of Zn in plants decreases growth, but excess Zn has significant toxicity to biological systems through metal-based cytotoxic reactions. Therefore, the uptake and transport of Zn must be strictly regulated. Intracellular Zn homeostasis is achieved through the coordinated regulation of specific transporters engaged in Zn influx, efflux, and intracellular compartmentalization. Gramineous plants produce and secrete mugineic acid family phytosiderophores (MAs) to acquire iron (Fe) from the soil. Furthermore, MAs chelate various divalent cations, including Zn. In rice, MAs plays a role in the translocation of Zn within plants. Nicotianamine (NA), biosynthetic precursor of MAs, is thought to be one of the principal metal chelators, including Fe and Zn, in all higher plants. In addition to the central role of NA, MAs in gramineous plants are thought to be involved in Fe and Zn translocation.

## METHODS

Using promoter- $\beta$ -glucuronidase (GUS) analysis, we investigated the expression patterns during rice seed development and germination of the genes *OsNAS1*, *OsNAS2*, *OsNAS3*, *OsNAAT1*, and *OsDMAS1*, which encode enzymes that participate in the biosynthesis of MAs.

To investigate the metal ion flow during rice seed germination, the imaging of endogenous elements (Fe, Mn, Zn and Cu) was carried out using Synchrotron-based X-ray microfluorescence ( $\mu$ -XRF) at Spring-8.

## RESULTS AND DISCUSSION

*OsNAS1* and *OsNAS3* were expressed in the flower and the developing seed. 30 days after fertilization, *OsNAS1* expression was high in the endosperm and *OsNAS3* expression is high in the vascular bundles. The expression of *OsNAAT1* and *OsDMAS1* was also observed in the flower and the maturing seeds. At the late stage, expressions were high in the embryo. These results showed that NA and MAs are produced during the reproductive stage and may function in metal homeostasis in developing seed.

NA biosynthesis is thought to be active in the endosperm during germination because *OsNAS1* is strongly expressed at this site. These results strongly suggest that NA is required for the transport of various metal cations, including Fe and Zn, during seed germination in rice.

In this study, the distribution change in the seeds following to germination of each element was clearly observed by in vivo monitoring by the  $\mu$ -XRF. It was found that the Fe, Mn, Zn and Cu

were localized in the endosperm and embryo of the rice seeds and distribution change patterns during germination were different from each other.

## CONCLUSIONS

Our results suggest that NA and MAs have an important role in the distribution of mineral nutrients within the rice plant. In particular, during seed development, NA and MAs have a critical role in Zn and Fe accumulation in rice seeds. Therefore, enhancement NA and DMA biosynthesis results in the increase in bioavailable Zn and Fe contents in rice seeds. This may contribute to improve malnutrition in the asia via the supply of Zn and Fe rich staple food.

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## REFERENCES

- Ishimaru Y., Bashir K. and Nishizawa N. K. (2011). Zn uptake and translocation in rice plants. *Rice* 4:21-27
- Ishimaru, Y., Suzuki, M., Kobayashi, T., Wada, Y., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N. K. (2005) OsZIP4, a novel zinc-regulated zinc transporter in rice. *J. Exp. Bot.* 56: 3207-3214
- Nozoye T., Takahashi M., Kitajima N., Fukuda N., Hokura A., Terada Y., Nakai I., Ishimaru Y., Kobayashi T., Nakanishi H. and Nishizawa, N. K. (2009) In vivo analysis of metal distribution and expression of metal transporters in rice seed during germination process by microarray and X-ray Fluorescence Imaging of Fe, Zn, Mn, and Cu. *Plant & Soil* 325:39-51
- Nozoye T., Inoue H., Takahashi M., Ishimaru Y., Nakanishi H., Mori S. and Nishizawa, N. K. (2007) The expression of iron homeostasis-related genes during rice germination. *Plant Mol. Biol.* 64: 35-47
- Suzuki, M., Tsukamoto, T., Inoue, H., Watanabe, S., Matsuhashi, S., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N. K. (2008) Deoxymugineic acid increases Zn translocation in Zn-deficient rice plants. *Plant Mol. Biol.* 66: 609-617
- Suzuki, M., Takahashi, M., Tsukamoto, T., Watanabe, S., Matsuhashi, S., Yazaki, J., Kishimoto, N., Kikuchi, S., Nakanishi, H., Mori, S. and Nishizawa, N. K. (2006) Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. *Plant J.* 48: 85-97