Metal nutrients, such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu), are essential for normal plant growth. During rice seed germination, the nutrients stored in the seed are used for germination. The rice seed is composed mainly of embryo and endosperm and the metal nutrients are stored in both structures. An adequate flow of metal elements during seed germination is important for normal growth, but our understanding about the flow dynamics of these metal nutrients during rice seed germination is very limited. Free Fe ion is extremely toxic to plants, as it injures cells by catalyzing the generation of cellular free radicals. Therefore, small-molecule chelators have been speculated to be required for the utilization of the Fe. Mugineic acid family phytosiderophores (MAs) are natural Fe chelators that graminaceous plants secrete from their roots to solubilize Fe in the soil. As MAs have been identified in the xylem and phloem of rice and barley, MAs may play an important role in the long-distance transport of Fe in graminaceous plants as well. Nicotianamine (NA), an intermediate in the MA biosynthetic pathway, is also thought to be involved in the long-distance transport of metal cations in the plant body. Moreover, NA has been suggested to play an essential role in metal translocation and accumulation in developing seeds, based on analysis of NA-deficient transgenic tobacco (*Nicotiana tabacum*) plants. Rice produces and secretes deoxymugineic acid (DMA), the initial compound synthesized in the MA biosynthetic pathway.

We recently suggested that DMA and NA are involved in Fe transport during rice seed germination based on results from promoter-GUS and microarray analysis. Synchrotron-based X-ray microfluorescenece (μ-XRE) is a technique well suited to the localization of essential elements in cells and tissues. Most inorganic element biochemistry studies rely to some extent on bulk analysis of the elements of interest. Direct chemical element imaging is often more reliable than bulk analysis because it is not affected by sample preparation, which may alter metal distribution. In addition, chemical element imaging enables us to correlate tissue distribution with biochemical functions, or alteration of these functions. Trace element analysis requires the use of a technique that has detection limits as low as a few micrograms per gram. Direct chemical element imaging has unprecedented detection limits compared to more conventional chemical imaging using electron microscopy (>100 μg/g). In addition, direct chemical element imaging can detect all elements, which is not possible using radioisotopes. To examine metal flow during rice seed germination, we performed microarray analysis using mRNA extracted from germinating rice seeds, and found that many kinds of transporter genes involved in metal transport were strongly expressed and that their expression levels changed during seed germination. Furthermore, we examined the localization of the endogenous elements (Fe, Zn, Mn, and Cu) in rice seeds during germination using synchrotron-based X-ray microfluorescence (μ -XRF) at the Super Photon ring-8 GeV (SPring-8) facility.

Rice seeds were removed from agar plates without nutrients and sliced 12, 24, and 36 h after sowing. The samples were freeze-dried and used for X-ray imaging. Twelve hours after sowing, Fe accumulated in the dorsal vascular bundle, aleurone layer, and the endosperm. In the embryo, Fe accumulated in the scutellum facing the endosperm near the ventral vascular bundle and the vascular bundle of the scutellum 12 h after sowing. Twenty-four hours after sowing, Fe was still detectable in the dorsal vascular bundle. In the embryo, Fe distribution was dispersed in the scutellum, and had accumulated in the coleoptile. Fe accumulation in the epithelium and endosperm near the scutellum was also observed. Thirty-six hours after sowing, Fe was detected in the root tips. In the embryo, Fe was observed not only in epithelium, scutellum, and coleoptile, but also in the leaf primordium and radicle.

Zn was most abundant in the embryo. Zn was also distributed in the endosperm and was most abundant in the aleurone layer. After sowing, Zn in the endosperm decreased compared to Zn in the embryo. In the embryo, high levels of Zn accumulated in the radicle and leaf primordium. Twenty-four hours after sowing, Zn accumulation increased in the scutellum and the vascular bundle of the scutellum. In the scutellum, Zn accumulated in the endosperm similarly to Fe. After 36 h, Zn was distributed in the leaf primordium and the root tip. Zn was also detected in a specific area that was assumed to be the junction between the embryo and the dorsal vascular bundle.

Mn was accumulated in the endosperm and embryo. In the embryo, Mn accumulation in the scutellum decreased after sowing, whereas accumulation in the coleoptile increased. Thirty-six hours after sowing, Mn was also observed in the root tip.

Cu is also an important element for plants, but its concentration in the plant body is extremely low. Using the SPring-8 facilities, we succeeded in detecting Cu in the rice seed. Cu was detected not only in the embryo but also in the endosperm. After sowing, Cu in the scutellum decreased and accumulation in the coleoptile and root were observed. Using μ -XRE analysis at the SPring-8 facility, we have for the first time succeeded in documenting the changes in distribution of Fe, Mn, Zn, and Cu during rice seed germination. Changes in distribution showed distinct patterns between the elements. Several regulatory mechanisms were suggested to exist for metal homeostasis during rice seed germination.