Enhanced Content and Bioavailability of Zinc in the Endosperm of Rice Grain Following Activation-Tagging of Nicotianamine Synthase

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INTRODUCTION

Zinc (Zn) and iron (Fe) deficiencies in human populations are widespread problems, particularly in the developing world. This has stressed the need for biofortification of the cereal grain with Zn and Fe. Successful biofortification strategies must target not only the total concentrations of Zn and Fe, but also their bioavailability, i.e. their binding to different compounds in the grain. The objective of the present work was to investigate if over-expression of genes involved in the biosynthesis of nicotianamine, a key ligand involved in metal transport and homeostasis in plants, can be used to increase the content and bio-availability of Zn and Fe in the endosperm of the rice grain.

METHODS

Activation tagging of nicotianamine synthase genes *NAS2* and *NAS3* was used to generate rice lines with increased content of nicotianamine (Lee at al. 2009, 2011). In order to elucidate the chemical speciation of Zn and Fe, the rice grains were milled, and the remaining endosperm was extracted in an aqueous buffer prior to analysis by size exclusion chromatography hyphenated to an inductively coupled plasma mass spectrometer (SEC-ICP-MS; Persson et al. 2009). The ligands associated with Zn and Fe were identified following collection, lyophilization and re-injection of the heart-cut elemental peaks onto a hydrophilic interaction liquid chromatography (HILIC) column, coupled to electro spray ionization time of flight mass spectrometry (ESI-TOF-MS). The bioavailability of Zn and Fe in the seeds of the activation tagged lines was finally tested in a feeding experiment employing Zn or Fe deficient mice (Lee at al. 2009, 2011).

RESULTS AND DISCUSSION

Rice seeds harvested from the activation-tagged rice plants contained elevated amounts of Zn (2.2-fold), Fe (2.9-fold) and nicotianamine (9.6-fold). Compared to the wildtype, the seeds of the activation-tagged lines contained 16-fold more Zn and 7-fold more Fe bound to a low molecular weight complex. The ESI-TOF-MS mass spectrum confirmed that the main compound found in the collected Zn and Fe peaks indeed was nicotianamine. In addition, deoxymugineic acid which traditionally is regarded as a phytosiderophore in grasses, was present. Since deoxymugineic acid follows the same biosynthetic pathway as nicotianamine it is not surprising to observe both compounds. Mice fed with seeds from the activation-tagged lines recovered more rapidly from Zn or Fe deficiency than did control mice receiving wildtype seeds.

Seedlings of the activation tagged lines showed increased tolerance to Fe and Zn deficiencies as well as to Zn, copper (Cu) and nickel (Ni) toxicities. When exposed to excess cadmium (Cd) or lead (Pb), wildtype and activation-tagged seedlings did not differ in growth or in concentrations of heavy metals in shoots, roots and grains. In mature seeds, the content of Cu and Ni associated with the nicotianamine pool was 9.7- and 2.4-fold, respectively, higher than in the wildtype. However, this pool did not contain more Cd. Zn bio-fortification may thus be achieved without an accompanying increase in Cd content and bioavailability.

The expression of genes involved in Zn-uptake and biosynthesis of phytosiderophores was increased in the activation-tagged plants suggesting that the higher amount of nicotianamine led to greater exudation of phytosiderophores from the roots, as well as stimulated the uptake, translocation and seed-loading of Zn.

While activation of a single *NAS* gene drastically improved the amount of bio-available Zn and Fe in the endosperm of rice grains, the same was not the case following ectopic overexpression driven by the constitutive *ubiquitin* promoter. By activation-tagging, only native *NAS* genes are enhanced at their specific locations in the root pericycle or in phloem companion cells (Inoue et al. 2003). This targeted approach seems to overcome some of the bottlenecks in Zn loading into the grain, while a more systemic increase of nicotianamine biosynthesis may create unbalances in Zn handling within the plant. This knowledge is important for the design of future strategies for bio-fortification by biotechnology and plant breeding.

CONCLUSIONS

Our results demonstrate that not only the content but also the bioavailability of Zn in rice grain can be improved by activation-tagging of nicotianamine synthase genes. This biotechnological approach may prove to be a successful strategy in the battle against global Zn and Fe malnutrition.

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REFERENCES

Inoue, H., Higuchi, K., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2003) Three rice nicotianamine synthase genes, OsNAS1, OsNAS2 and OsNAS3, are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. *Plant J.* 36: 366–381.

Lee, S., Jeon, U.S., Lee, S.J., Kim, Y.K., Persson, D.P., Husted, S., Schjoerring, J.K., Kakei, Y., Masuda, H., Nishizawa, N.K. and An, G. (2009) Iron fortification of rice through activation of the nicotianamine synthase gene. *Proc. Natl. Acad. Sci. USA* 106: 22014-22019.

Lee, S., Persson, D.P., Hansen, T.H., Husted, S., Schjoerring, J.K., Kim, Y.-S., Jeon, U.S., Kim, Y.-K., Kakei, Y., Masuda, H., Nishizawa, N. and An, G. (2011) Bio-available zinc in rice is increased by activation tagging of *nicotianamine synthase*. *Plant Biotech. J.* doi: 10.1111/j.1467-7652.2011.00606.x (in press).

Persson, D.P., Hansen, T.H., Laursen, K.H., Schjoerring, J.K. and Husted, S. (2009) Simultaneous iron, zinc, sulphur and phosphorus analysis of barley grain tissues using SEC-ICP-MS and IP-ICP-MS. *Metallomics* 1: 418-426.