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# **Title**

Over-expressing a barley ZIP gene doubles grain zinc content in barley (Hordeum vulgare)

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#### Introduction

Zinc (Zn) is an essential micronutrient for all organisms. Micronutrient malnutrition affects more than half of the world's population, especially women and preschool children (Welch and Graham, 2004). Zn and Vitamin A malnutrition were considered to be the most important global challenges at the Copenhagen Consensus 2008 conference (www. copenhagenconsensus.com). Biofortification of staple food crops, such as the grain of wheat and rice, has become an important strategy to alleviate micronutrient deficiencies in humans (Palmgren et al., 2008). At least three gene families are involved in plant Zn homeostasis (Gaither and Eide, 2001). Metal transporters of the ZRT, IRT-related protein (ZIP) family have recently been identified as the main transporters controlling Zn influx into the cytoplasm (Gaither and Eide, 2001; Palmgren et al., 2008). The heavy metal transporter ATPase (HMA) family is involved in xylem Zn loading. The metal tolerance protein (MTP) family is involved in vacuolar Zn compartmentalisation. So far little is known about the transporters responsible for loading Zn into grain (Palmgren et al., 2008). A greater understanding of the effects of the genes involved in Zn transport in cereal plants, particularly for Zn translocation into the grain, is important for genetic enhancement of grain Zn content in the major staple crops. In this study, we generated transgenic barley plants over-expressing a barley ZIP gene, HvZIP7 to evaluate its effect on grain Zn loading.

#### Results and discussion

We isolated an ortholog of *OsZIP7* from barley, and named this barley ortholog as *HvZIP7*. Transcript profiles of *HvZIP7* and sub-cellular localization of HvZIP7 suggest that it plays a role in Zn translocation in the plant. To determine the potential role of *HvZIP7* in grain Zn loading, transgenic barley lines over-expressing *HvZIP7* were generated. Homozygous single-copy, transgenic barley lines were selected for the analysis of over-expression of *HvZIP7*. When transgenic barley lines were grown in a low Zn supply, there was no difference in grain Zn concentration and content between transgenic and the null lines or wild type. When a low dose of Zn was supplemented during anthesis, the grain Zn concentration and content were significantly increased in both null and transgenic line relative to those without Zn supplement. However, the transgenic line with Zn supplement had much higher grain Zn concentration and content than the null line. The grain Zn concentration and content in the transgenic plants were increased by over 50% relative to the null plants. The concentration and content of other micronutrients such as Fe, Mn and Cu in the grain were similar between the transgenic lines and the null lines or the wild type.

When the transgenic lines and controls were grown in a high Zn supply, grain Zn concentration and content in the transgenic lines were doubled relative to those of the null lines and wild type. The concentration and content of the other micronutrients in grain were comparable between transgenic and null plants or wild type.

Our results showed that the over-expression of *HvZIP7* in barley specifically increased grain Zn content, and has little effect on Fe, Mn and Cu under low or high supply of Zn. The grain Zn concentration in the low Zn supply is comparable to that of barley plants grown in a Zn-deficient field site with Zn fertilization (Graham et al., 1992), which suggests that transgenic lines have potential to increase grain Zn content by 50% in the field-grown plants with standard Zn fertilization. A further increase in grain Zn content is possible if a higher rate of Zn fertilizers is applied.

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