

Isolation and Characterization of *Lotus japonicus* Genes Involved in Iron and Zinc Homeostasis

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INTRODUCTION

Legumes are important staples in the developing world. They are frequently grown in soil with limited nutrient availability. There is a natural variation of seed micronutrient content among legumes of the same species even when plants are grown under similar conditions (Mohamed et al. 1991). This indicates that a genetic factor affects the nutritional value of these staples. Consequently, it is possible that the micronutrient value of seeds is reduced during breeding for specific traits. The analysis of progeny from each cross for micronutrient content is very tedious and costly. Therefore, it is important to have genetic markers for high micronutrient traits during the selective breeding process.

Plants use finely tuned mechanisms to keep appropriate levels of Fe and Zn in each of their organs. Several genes involved in Fe and Zn homeostasis have been described in yeast, and a few orthologs have been studied in plants (Grotz and Guerinot 2006). The goal of this project was to find ways to improve the nutritional value of legumes by identifying genes and proteins important for Fe and Zn regulation in the model legume *Lotus japonicus*. The results obtained from *L. japonicus* will be used to develop genetic markers for the trait of high micronutrient content in common bean. The use of these genetic markers can reduce the time and cost required to develop new cultivars with improved nutritional content.

METHODS

Two approaches were applied for the identification of genes that are important for micronutrient homeostasis. We used gene sequences that are known to play a role in Fe and Zn metabolism in different organisms to search for expressed sequence tags (ESTs) and genomic loci of *L. japonicus*. Identified sequences were used for gene regulation studies. Furthermore, the micronutrient content of different tissues from four *Lotus* ecotypes/species (*L. japonicus* Gifu, *L. japonicus* Miyakojima (MG-20), *L. burttii*, and *L. filicaulis*) was analysed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). We performed mapping and quantitative trait loci (QTL) analysis of recombinant inbred lines (RIL) from a cross between *L. japonicus* Gifu and *L. filicaulis*.

RESULTS AND DISCUSSION

We identified *L. japonicus* ESTs and genomic loci corresponding to ferritins, ferric reductases, adenosine triphosphatases, ferritin transcription factors, inosine triphosphate, metal transport proteins of the ZIP family, and cation transporters of the natural resistance associated macrophage protein (NRAMP) family.

The content of Fe and Zn in the seeds of the two analysed *L. japonicus* ecotypes and *L. burttii* were similar. Significantly different levels of Fe and Zn were found between *Lotus filicaulis* and *L. japonicus* Gifu (Fig. 1).

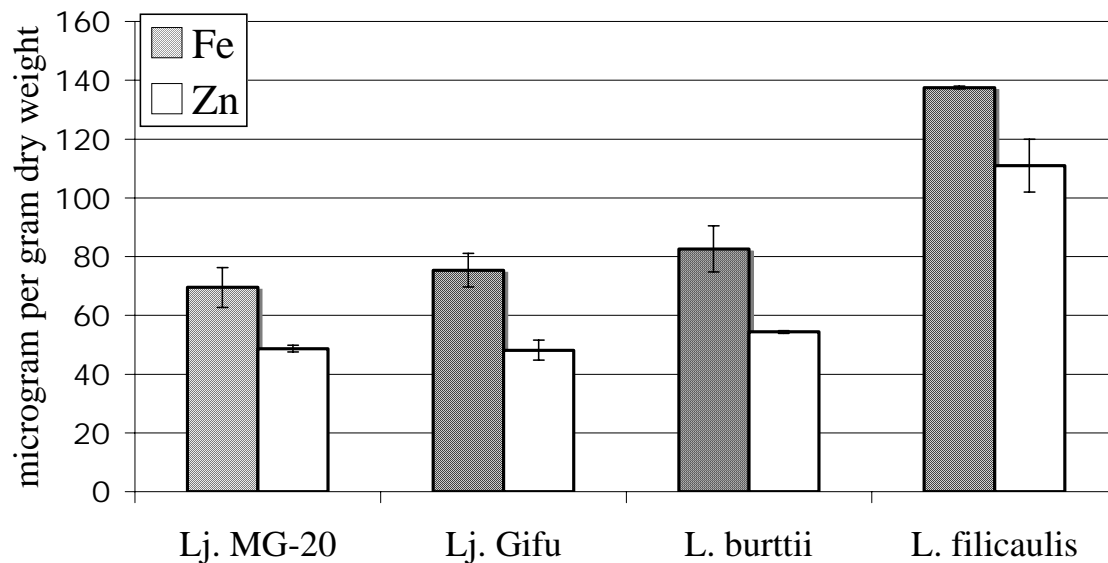


Fig. 1. Fe and Zn content in seeds from *Lotus japonicus* ecotypes MG-20 and Gifu, *Lotus burttii* and *Lotus filicaulis*.

These results were used to map genes important for Fe and Zn homeostasis using *L. filicaulis* x *L. japonicus* Gifu recombinant inbred lines. Preliminary results indicated the presence of two main QTL for Zn and one QTL for Fe. Furthermore, the main QTL for Fe corresponded to one of the QTL for Zn.

CONCLUSIONS

Sequences with high similarity to most of the known genes involved in Fe and Zn homeostasis were identified in the model legume *Lotus japonicus*. The presence of main QTL for Fe and Zn in the model legume indicates that it will be possible to monitor high Fe and Zn traits using genetic markers during plant breeding. It is possible that a single locus is influencing both Zn and Fe content.

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