

# Identification and Comparison of Quantitative Trait Loci Associated with Seed Zinc Concentration in *Lotus japonicus* and *Medicago truncatula*

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## INTRODUCTION

Reports indicate that Zn deficiency is a widespread problem for crop plants and humans. Increasing the amount of bioavailable micronutrients such as Fe and Zn in plant foods for human consumption is a challenge especially in developing countries where plant foods comprise a significant portion of the diet. Legumes are one of the largest and economically most important plant families. Legume seeds are a rich source of proteins and have the potential to provide the essential nutrients required by humans who subsist on a plant based diet. However, concentration and bioavailability of nutrients in these seeds are limited when compared to animal food products (Wang et al. 2003). Our research program is interested in increasing the nutrient concentration and availability of plant based protein sources to improve human nutrition. To improve seed nutrient concentrations, we have to understand the genetic basis and identify molecular components involved in nutrient distribution.

Genetic tools such as recombinant inbred lines (RILs) can be used to identify quantitative trait loci (QTLs) associated with seed nutrient traits. Quantitative trait loci have been successfully used in a number of other model species to identify genes of interest for studying seed nutrient homeostasis (Vreugdenhil et al. 2004, Panthee et al. 2006). By identifying loci that are involved in the uptake and storage of nutrients in seeds, we can create mapping markers for related crop species of interest and improve the seed nutrient concentration in more economically important members of *Leguminosae*.

We are conducting research to identify QTLs linked to a number of nutritive qualities including improved seed Zn concentration in the model legume species *Lotus japonicus* and *Medicago truncatula*. Both species, *L. japonicus* and *M. truncatula*, are the focus of international genome sequencing efforts, and a number of scientific resources have been created and released to the scientific community at large to further study members of this family.

## METHODS

To identify loci that influence seed nutrient concentrations, 95 lines of *M. truncatula* RIL population (Jemalong x DZA315.16) were grown for seed harvest and subsequent nutrient analysis using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The QTL Cartographer software (v. 2.5; N.C. State University, Bioinformatics Research Center) was used to identify QTLs linked to 93 previously mapped molecular markers using composite interval mapping. A LOD threshold between 2.5 and 2.7 was used to report a significant QTL for all traits. The LOD score threshold was determined using the permutation test (1000 repetitions) at a *p* value of 0.05 for normally distributed data.

Work to identify the QTLs associated with seed Zn content will be conducted with *L. japonicus* similar to *M. truncatula* using 120 lines of the *L. japonicus* RIL population (Gifu B-129 x Miyakojia MG-20).

Following identification of QTLs associated with seed Zn concentration, comparative mapping efforts will be done to assess syntenic relationships between these QTLs in both species.

## RESULTS AND DISCUSSION

In *M. truncatula*, frequency distribution of seed Zn showed transgressive segregation in the RIL population. There were no correlations between Zn and other nutrient concentrations. Significant Zn QTLs were found on chromosomes 4, 7, and 8 with 24.2, 17.9, and 8.9% of explained variance, respectively. These QTLs overlapped with QTLs for other nutrients. For example, the Zn QTL on chromosome 7 overlapped with a significant Fe QTL which also had a high level of explained variance (21.2%). Additional lines were grown and are currently analysed for seed nutrients.

Nutrient analysis is currently conducted on seeds of *L. japonicus* RILs.

## CONCLUSIONS

Transgressive segregation for seed nutrient concentrations demonstrated that new allelic combinations can be used to increase seed Zn levels. For loci where different nutrient QTLs co-localize, it is possible that a common whole-plant phenotypic trait such as transpiration efficiency and root size might contribute to seed nutrient levels.

A number of studies comparing members within the legume family have identified regions of synteny at macro and micro levels (Choi et al. 2004a, 2004b; Cannon et al. 2006). Comparative mapping between these two legume species will enable us to assess the level of co-linearity for Zn-associated loci between the two genomes.

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## REFERENCES

- Cannon, S.B., McCombie, W.R., Sato, S., Tabata, S., Denny, R., Palmer, L., Katari, M., Young, N.D. and Stacey, G. (2003) Evolution and microsynteny of the apyrase gene family in three legume genomes. *Mol. Genet. Genomics* 270: 347–361.
- Choi, H.K., Kim, D., Uhm, T., Limpens, E., Lim, H., Mun, J.H., Kalo, P., Penmetsa, R.V., Seres, A. and Kulikova, O. (2004a) A sequence-based genetic map of *Medicago truncatula* and comparison of marker colinearity with *M. sativa*. *Genetics* 166: 1463–1502.
- Choi, H.K., Mun, J.H., Kim, D.J., Zhu, H., Baek, J.M., Mudge, J., Roe, B., Ellis, N., Doyle, J. and Kiss, G.B. (2004b) Estimating genome conservation between crop and model legume species. *Proc. Natl. Acad. Sci. USA* 101: 15289–15294.
- Panthee, D.R., Pantalone, V.R., Sams, C.E., Saxton, A.M., West, D.R., Orf, J.H. and Killam, A.S. (2006) Quantitative trait loci controlling sulfur containing amino acids, methionine and cysteine, in soybean seeds. *Theor. Appl. Genet.* 112: 546-553.
- Vreugdenhil, D., Aarts, M.G.M., Koornneef, M., Nelissen, H., and Ernst, W.H.O. (2004) Natural variation and QTL analysis for cationic mineral content in seeds of *Arabidopsis thaliana*. *Plant Cell Environ.* 27: 828-839.
- Wang, T.L., Domoney, C., Hedley, C.L., Casey, R. and Grusak, M.A. (2003) Can we improve the nutritional quality of legume seeds? *Plant Physiol.* 131: 886-891.