Variation in Zinc-Deficiency Tolerance in a RIL Population of *Arabidopsis thaliana* and Determination of QTLs Underlying this Variation

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INTRODUCTION

Zinc deficiency is a worldwide problem that results in significant losses in crop yield and has detrimental effects on quality. It is also a well-documented nutritional problem in human beings, especially in the developing world, leading to severe health complications such as mental and growth retardation, delayed sexual maturation, immune disorders, dermal problems, impaired wound healing and anorexia.

Cereals, especially wheat and rice, are major staple foods that are inherently low in Zn and widely cultivated in Zn-deficient soils. Efforts aimed at enhancing the Zn-deficiency tolerance of major crop plants will significantly contribute to global crop production, and improvements in Zn concentrations of cereal grains will result in important benefits to human health.

Significant genotypic variations in Zn-deficiency tolerance and Zn accumulation in seeds are known to exist among different cultivars of major crop plants. These natural variations are being utilized in conventional and modern breeding programs, but there is still a lack of knowledge of molecular mechanisms affecting Zn-deficiency tolerance and Zn accumulation in grain.

In this project, we conducted a large screening study by using an *A. thaliana* Recombinant Inbred Line (RIL) population, which was developed from a cross between “Landsberg erecta (Ler)” and “Cape Verde Islands (Cvi)” to evaluate the genotypic variation in Zn-deficiency tolerance and Zn accumulation in seeds. Initially, we focused on Zn-deficiency tolerance. The data will be used for the identification of quantitative trait loci (QTLs) involved in tolerance to Zn deficiency.

METHODS

The parents, Ler and Cvi, and 160 RILs were grown on a Zn-deficient calcareous soil from Central Anatolia. The soil was amended with essential nutrients, including N, P, K, S and Fe for this screening study using 3 replicates. Plants were grown with low (e.g., 0.25 mg kg^{-1} Zn) and adequate (e.g., 3 mg kg^{-1} Zn) Zn treatments under greenhouse conditions. After 5 weeks of growth, shoots were harvested, dried and measured for dry matter production.

The severity of Zn-deficiency symptoms were evaluated for each line by giving symptom scores prior to harvest. The tolerance index (%) was calculated by dividing the shoot dry weight at low Zn supply by shoot dry weight at adequate Zn conditions. Shoot Zn concentration and the concentrations of other relevant nutrients were measured using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES).

Finally, the collected physiological data will be used to identify QTLs that can explain the observed variations.

RESULTS AND DISCUSSION

A preliminary screening study was completed. There was substantial variation in tolerance to Zn deficiency among the lines of the RIL population tested. The severity of chlorosis due to Zn deficiency, and the tolerance index were utilized to evaluate the differential tolerance. Table 1 shows the average shoot dry weights and tolerance indices of RILs grouped together with respect to their symptom scores. The average tolerance index increased significantly as the symptoms of Zn deficiency become less severe. Tolerance indices of individual RILs varied between ca. 21% and 98%.

Table 1. Genetic variation in Zn-deficiency tolerance as reflected by the tolerance index.

Symptoms of Zn deficiency*	Shoot dry weight		Tolerance Index**
	- Zn	+ Zn	
	(mg plant-^{1})		(%)
1 (n=18)	5.5 ± 3.0	12.8 ± 5.4	44.5
2 (n=43)	6.5 ± 2.5	13.5 ± 5.6	52.9
3 (n=29)	6.0 ± 2.1	10.8 ± 5.5	63.5
4 (n=54)	8.5 ± 3.0	10.8 ± 5.8	95.2
Mean	6.6	12.0	64.0

* 1- very severe, 2- severe, 3- mild, and 4-slight or absent.

** Tolerance Index = (shoot dry weight at -Zn / shoot dry weight at +Zn) x 100.

Shoot Zn concentrations of the lines in the Ler/Cvi RIL population also exhibited a significant variation. Table 2 shows the relation between symptom severity and Zn concentration.

Table 2. Variation in shoot Zn concentration under deficient and control conditions.

Symptoms of Zn deficiency*	Zn concentration		Zn content	
	- Zn	+ Zn	- Zn	+ Zn
	(mg kg^{-1} dry wt.)		(ng plant^{-1})	
1 (n=18)	22.31 ± 10.28	58.60 ± 11.00	189 ± 137	637 ± 294
2 (n=43)	19.29 ± 7.80	61.08 ± 13.44	145 ± 95	668 ± 377
3 (n=29)	19.75 ± 7.24	57.49 ± 12.70	137 ± 77	741 ± 377
4 (n=54)	15.25 ± 4.03	59.47 ± 14.96	92 ± 49	725 ± 442
Mean	19.2	59.2	140.8	692.8

* 1- very severe, 2- severe, 3- mild, and 4-slight or absent.

These are data from our preliminary screening experiment. A similar screening study is in progress. The results of both screening tests will be used to identify QTLs. Results of the QTL studies will be presented and discussed in the conference together with the physiological data.

CONCLUSIONS

The Ler/Cvi RIL population exhibited a significant variation in tolerance to Zn deficiency and in shoot Zn concentration, which could be exploited for an understanding of the genetic basis of Zn-deficiency tolerance. QTL studies will reveal genomic regions that are involved in tolerance to Zn deficiency. Future studies will also focus on Zn accumulation in seeds.