Identification of Differentially Expressed Zn-Related Genes in *Triticum dicoccoides* Accessions under Zn Deficiency

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INTRODUCTION

Zinc deficiency is particularly prevalent in wheat crops leading to significant reductions in yield and nutritional quality of grains. People living in developing countries are severely affected by Zn deficiency mostly due to farming systems that do not produce adequate micronutrients to meet human needs (Welch 2005). It was found that Zn bioavailability in wheat is very low in Central Anatolia where soils are calcareous and Zn-deficient (Cakmak et al. 1999). Currently, the molecular pathways involved in differential expression of Zn-deficiency tolerance are unclear. Increasing evidence is available showing that wild wheat exhibits a significant variation in tolerance to Zn deficiency. In this study, different wild wheat genotypes were studied for their Zn deficiency tolerance. Plants were grown under different Zn concentrations, and differential expression of genes was examined using complementary DNA-Amplified Fragment Length Polymorphism (cDNA-AFLP) analysis.

MATERIALS AND METHODS

In this study, 23 accessions of wild tetraploid wheat (*Triticum dicoccoides*) were used. Plants were grown in a greenhouse in a Zn-deficient soil from Central Anatolia with (+Zn = 5 mg Zn kg^{-1} soil) and without (-Zn = 0) Zn treatments. Based on the Zn-efficiency values (ratio of dry matter production at -Zn to dry matter production at +Zn) of genotypes tested, the most tolerant and susceptible genotypes were identified to be used in molecular studies. Six wild tetraploid wheat genotypes were selected and grown in hydroponic solutions under the following Zn fertilization conditions: -Zn (severe Zn deficiency), 10^{-8} M Zn (moderate Zn deficiency), 10^{-7} M Zn (slight Zn deficiency), 10^{-6} M Zn (adequate Zn supply), 10^{-4} M Zn (toxic Zn supply). After growing for 14 days, plants were harvested, frozen in liquid nitrogen immediately and stored at –80 ºC. Shoot and root parts were separately harvested.

Gene-specific primers were designed for the *Triticum aestivum ZIP* messenger ribonucleic acid (*ZIP* mRNA) coding sequence (Accession AY864924) to amplify the corresponding region in wild wheat genotypes using polymerase chain reaction (PCR) analysis. The fragment of interests is currently cloned using pGEM-T Easy Vector Systems to be sequenced for the identification of different ZIP transporter alleles. The cDNA-AFLP analysis is applied to plants to determine differentially expressed up- and down-regulated genes in wild tetraploid wheat genotypes grown at different Zn concentrations. Gene expressions are quantified using real-time PCR analysis.

RESULTS AND DISCUSSION

Based on a preliminary screening test with 23 *Triticum dicoccoides* accessions, three wild wheat accessions (MM 5/4, MM 5/2, 24-39) were identified as tolerant, while the other three (18-60, 33-48 and 19-36) were observed as susceptible. Among the susceptible accessions, 33-48 and 19-36 were affected the most.

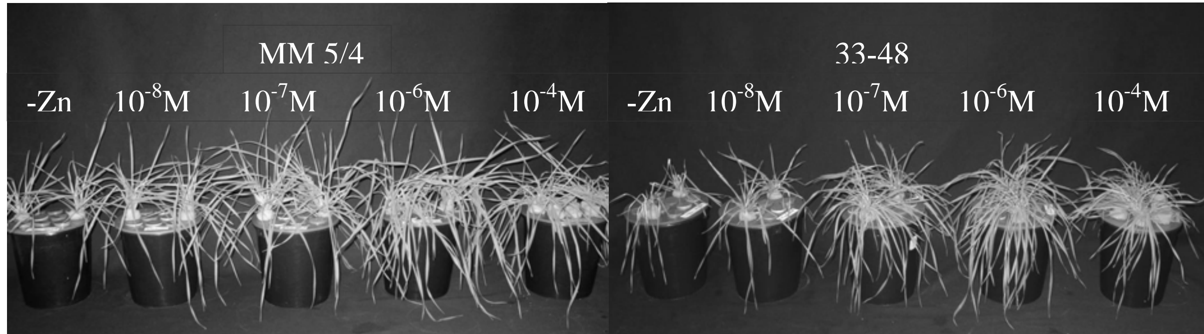

Fig. 1. Plants MM 5/4 (tolerant) and 33-48 (susceptible) grown for 14 d in nutrient solutions at differing Zn concentrations.

The expression of ZIP mRNA was studied in Zn-deficiency tolerant accessions using a Reverse Transcription (RT) PCR technique. The results showed that ZIP mRNA expression decreased with increasing Zn concentrations in the growth medium (Fig.2). It seemed that a reduction in Zn supply beyond 10^{-7} M Zn does not cause a further increase in ZIP expression suggesting that 10^{-7} M Zn is the critical dosage under the given conditions.

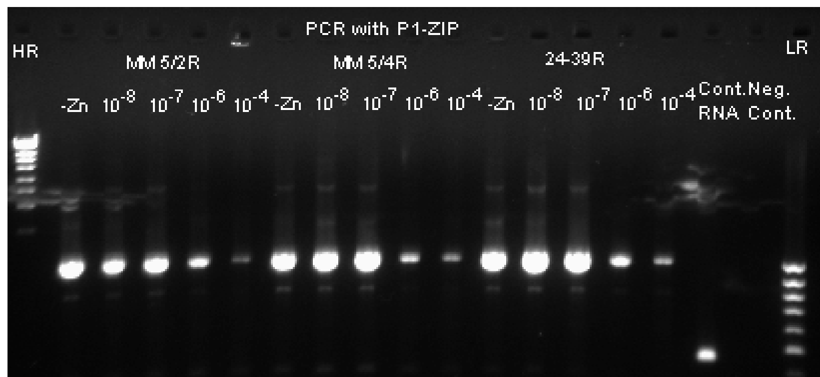

Fig. 2. RT-PCR Gel Picture of Root cDNA Samples of MM5/2, MM5/4 and 24-39 using designed ZIP primer.

Currently, the same approach is applied together with cDNA-AFLP analysis to other accessions differing in Zn-deficiency tolerance. Results will be presented at the conference.

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