

# Quantification of Gene Expression in *Agrostis* Grass Species Subjected to Zinc Deficiency

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## INTRODUCTION

Zinc is an essential mineral nutrient and a cofactor of enzymes and proteins involved in many cellular mechanisms (Marschner 1986). Zinc deficiency in soils causes severe reductions in crop production and grain quality, as shown in different countries like in India, Turkey, China and Australia (Alloway 2002).

Data on plant responses to Zn deficiency at the molecular level are lacking. Differential display (DD) technology is one of the major tools that are used to interpret genomic information. To speed up the identification of differentially expressed genes, DD was developed with the aim of overcoming limitations of previous methodologies. It is a simple, sensitive, systematic and reliable method to identify differentially expressed genes (Liang and Pardee 1992). In this study, this approach was used to quantify and to identify genes related to Zn-deficiency in grasses by applying messenger ribonucleic acid (mRNA)-DD and real-time polymerase chain reaction (PCR) techniques.

## METHODS

Six different grass species (Colonial Bentgrass, Creeping Bentgrass, Velvet Bentgrass, *Festuca rubra tricophylla*, Bermuda grass and Perennial Ryegrass) were grown in a Zn-deficient soil from Central Anatolia with (+Zn = 5 mg kg<sup>-1</sup> soil) and without (-Zn) Zn supply under greenhouse conditions. The total RNAs extracted from shoots were subjected to mRNA-DD method using 72 different DD primer combinations (Table 1). The PCR products were detected using agarose gel electrophoresis with ethidium bromide staining.

## RESULTS AND DISCUSSION

Six different grass species showed a large variation in tolerance to Zn deficiency (Fig. 1). Among the grass species tested, Perennial Ryegrass and Colonial Bentgrass exhibited the highest tolerance and sensitivity to Zn deficiency in soils, respectively.

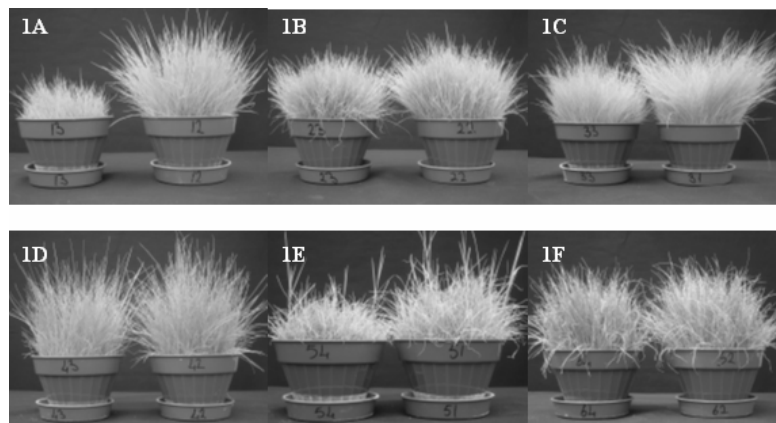
Zinc-related gene expression profiling in the Colonial, Creeping and Velvet Bentgrass was obtained using the mRNA-DD method. Eight candidate genes showing divergent profiles from the controls have been identified and sequenced for each species. The preliminary data obtained for Creeping Bentgrass indicated the existence of four candidate genes expressed under Zn deficiency. These genes were sequenced and searched using the Basic Local Alignment Search Tool (BLAST). The results showed that these genes have some short sequences similar to known genes of many plants, including *Oryza sativa* and *Arabidopsis thaliana*. All four candidate genes have open reading frames (ORF).

The candidate genes will be sequenced, further analysed and discussed. Full length complementary DNA (cDNA) of selected genes will be obtained using Rapid Amplification of cDNA Ends (RACE). Coding genes will be cloned to an expression vector for protein level analysis. Further experiments are ongoing, and the results will be presented and discussed at the conference.

**Table 1. mRNA Differential display primers.**

Table1  
Primers used in mRNA differential display

| Primer designation | Sequence (5' -3')                       |
|--------------------|---|
| P1                 | ATT AAC CCT CAC TAA ATG CTG GGG A       |
| P2                 | ATT AAC CCT CAC TAA ATC GGT CAT AG      |
| P3                 | ATT AAC CCT CAC TAA ATG CTG GTG G       |
| P4                 | ATT AAC CCT CAC TAA ATG CTG GTA G       |
| P5                 | ATT AAC CCT CAC TAA AGA TCT GAC TG      |
| P6                 | ATT AAC CCT CAC TAA ATG CTG GGT G       |
| P7                 | ATT AAC CCT CAC TAA ATG CTG TAT G       |
| P9                 | ATT AAC CCT CAC TAA ATG TGG CAG G       |
| T1                 | CAT TAT GCT GAG TGA TAT CTT TTT TTT TAA |
| T2                 | CAT TAT GCT GAG TGA TAT CTT TTT TTT TAC |
| T3                 | CAT TAT GCT GAG TGA TAT CTT TTT TTT TAG |
| T4                 | CAT TAT GCT GAG TGA TAT CTT TTT TTT TCA |
| T5                 | CAT TAT GCT GAG TGA TAT CTT TTT TTT TCC |
| T6                 | CAT TAT GCT GAG TGA TAT CTT TTT TTT TCG |
| T7                 | CAT TAT GCT GAG TGA TAT CTT TTT TTT TGA |
| T8                 | CAT TAT GCT GAG TGA TAT CTT TTT TTT TGC |
| T9                 | CAT TAT GCT GAG TGA TAT CTT TTT TTT TGG |



**Fig. 1. Effect of Zn application (-Zn: left; +Zn right) on growth of Colonial Bentgrass (1A), Creeping Bentgrass (1B), Velvet Bentgrass (1C), *Festuca rubra tricophylla* (1D), Bermuda grass (1E) and Perennial Ryegrass (1F) grown for 30 d.**

## REFERENCES

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