

Molecular and Physiological Characterization of Zinc Regulated Genes in Cultivated Wheat

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INTRODUCTION

Zinc deficiency is a widespread micronutritional problem in crop plants that affects crop production and quality severely. Wheat is particularly affected by Zn deficiency. The reductions in grain yield caused by Zn deficiency vary from 5 to 600% depending on soil type and country (Cakmak et al. 1999). The development of plant genotypes that tolerate Zn deficiency is generally accepted as a solution to the problem. The knowledge of molecular and physiological mechanisms contributing to Zn deficiency tolerance is essential for a successful breeding program that aims to develop genotypes with such a tolerance. However, data related to molecular mechanisms of Zn deficiency tolerance are very limited. Thus, the main emphasis of this study was to understand the molecular factors involved in expression of high Zn deficiency tolerance in wheat. Molecular techniques were applied to bread and durum wheat genotypes that differ greatly in their tolerance to Zn deficiency. The differential display technique was chosen because it is simple and provides a concurrent comparison of differentially regulated multiple ribonucleic acid (RNA) samples (Suzuki et al. 2001). The main objective of the study was to identify and to isolate differentially expressed genes in wheat genotypes when exposed to a range of Zn applications.

METHODS

Thirty three bread (*Triticum aestivum*) and durum (*Triticum turgidum*) wheat genotypes were grown on a Zn deficient soil with +Zn (2,5 mg kg⁻¹ soil) and -Zn applications under greenhouse conditions for 38 d. Afterwards, shoots were harvested and Zn efficiency values (dry weight at -Zn / dry weight at +Zn) were calculated. Based on Zn efficiency values and the severity of Zn deficiency symptoms, the most sensitive and the most tolerant 3 bread and 3 durum wheat genotypes were selected for subsequent molecular studies. Selected genotypes were grown in nutrient solution containing a wide range of Zn (0, 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁴M) for 15 d. At harvest, shoot and root samples were taken for isolation of RNA according to the Trizol isolation protocol. After c-DNA synthesis from the RNA templates, a polymerase chain reaction (PCR) was performed using differential display primers (e.g.; P and T primers). Additionally, a PCR was done using primers that are designed against the zinc regulated protein, ZIP of *Triticum aestivum*.

RESULTS AND DISCUSSION

We observed a large genetic variation in tolerance to Zn deficiency. In general, bread wheat genotypes showed higher tolerance to Zn deficiency than durum wheat genotypes. The genetic variation for Zn deficiency tolerance was very high among the genotypes of a given species especially in durum wheats (Table 1). The differential display PCR was first applied to Zn deficiency tolerant bread wheat genotype Alpu by using two different combinations of P and T primers (Fig. 1). Based on these results, it seemed that the expression profile is markedly affected by the Zn status of plants. Currently, this expression profile is being tested with Zn efficient and inefficient genotypes and selected results will be presented at the conference.

Table 1. Shoot dry matter production and Zn efficiency ratio (dry matter yield at -Zn / dry matter yield at + Zn)

Genotype	-Zn	+Zn	Zn efficiency ratio
Durum Wheat			
	(mg plant ⁻¹)		(%)
Selcuklu	657 ± 135	711 ± 176	92,4
Balcali 85	377 ± 22	436 ± 29	86,4
Meram	379 ± 14	455 ± 33	83,3
Gediz	286 ± 23	472 ± 62	60,5
C-1252	254 ± 54	460 ± 3	55,2
Kumbet 2000	205 ± 17	457 ± 7	44,9
Bread Wheat			
Bezostaya-1	544 ± 49	550 ± 30	98,9
Dagdas	474 ± 41	488 ± 41	97,1
Alpu 01	483 ± 24	508 ± 34	95,1
Ahmetaga	407 ± 155	558 ± 55	73,0
Karahan	546 ± 100	909 ± 56	60,1
Bagci	414 ± 12	694 ± 80	59,6

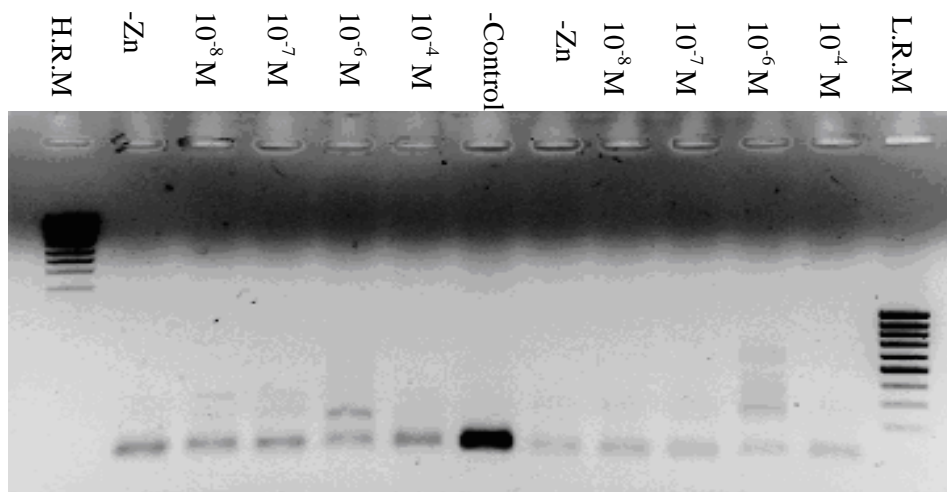


Fig. 1. Agarose gel showing differentially expressed c-DNA bands amplified with P3/T3 (e.g. ,lane 2-6) and P3/T2 (e.g. , lane 8-12) primer combinations upon exposure to different Zn levels. H.R.M refers to a high range marker, L.R.M refers to a low range marker.

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REFERENCES

- Cakmak, I., Kalayci, M., Ekiz, H., Braun, H.J. and Yilmaz, A. (1999) Zinc deficiency as an actual problem in plant and human nutrition in Turkey:a Nato-Science for Stability Project. *Field Crops Res.* 60: 175-188.
- Suzuki, N, Koizumi, N and Sano, H. (2001) Screening of cadmium-responsive genes in *Arabidopsis thaliana*. *Plant, Cell and Environment* 24:1177–1188.