

# The Molecular Basis for Zn Hyperaccumulation in *Thlaspi caerulescens*

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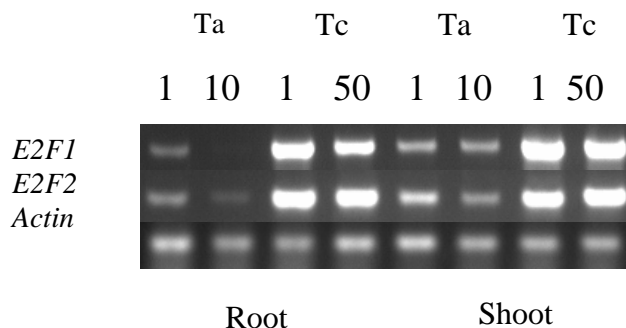
## INTRODUCTION

*Thlaspi caerulescens* (J&C Presl) is a Zn/Cd hyperaccumulator that tolerates extremely high levels of Zn and Cd in the soil, and accumulates both metals in the shoot to very high levels (Brown et al. 1995). The activity of several Zn transporters have already been shown to be altered in *T. caerulescens* compared with a related Zn non-accumulator species, *T. arvense*. For example, root-Zn influx was shown to be considerably greater in the hyperaccumulator species. However, the relative affinity of the root transporter for Zn is quite similar in both *Thlaspi* species, which led the authors to speculate that increased transporter expression was the basis for this increased Zn uptake (Lasat et al. 1996).

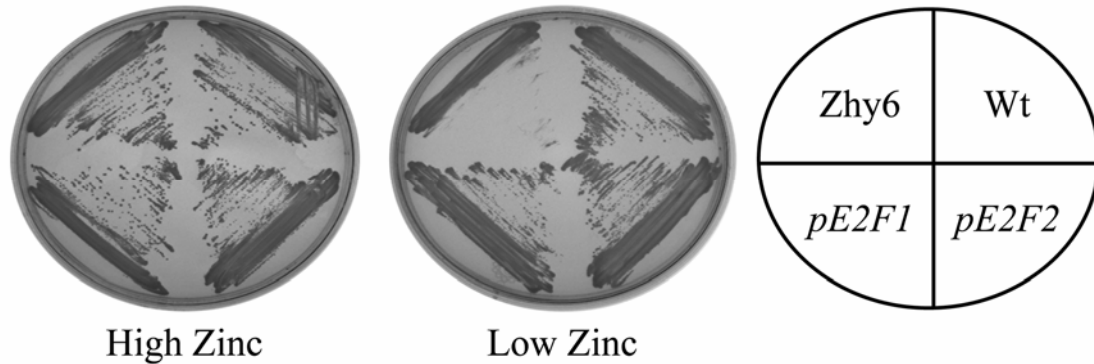
Subsequently, Zn transporters that are believed to be involved in many different aspects of Zn transport and homeostasis, such as the putative plasma membrane root transporter ZNT1, the vacuolar transporter MTP1, and the putative xylem loader HMA4, exhibited much higher gene expression, or hyperexpression, in *Thlaspi caerulescens* (Pence et al. 2000, Assunção et al. 2001, Papoyan and Kochian 2004). This hyperexpression phenotype is not solely a trait that *T. caerulescens* possesses. Another Cd hyperaccumulator, *Arabidopsis halleri*, exhibited also hyperexpression of multiple genes involved with Zn homeostasis (Becher et al. 2004, Weber et al. 2004). It remains unknown if one or several factors account for this modification in gene expression for a number of Zn transporters.

We have identified two transcription factors from the E2F family of cell cycle-related transcription factors, E2F1 and E2F2, which may be involved in the regulation of certain plant Zn transporter genes. Both of these transcription factors are hyperexpressed in *T. caerulescens* (Fig. 1). Furthermore, both transcription factors, when expressed in yeast, can replace the function of *ZAP1*, a yeast transcription factor that helps mediating the Zn-dependent regulation of the expression of a number of yeast Zn transporters that are closely related to the *T. caerulescens* Zn-transporter gene, *ZNT1*.

## RESULTS AND DISCUSSION



**Fig. 1.** Expression of E2F1 and E2F2 in *T. arvense* and *T. caerulescens* under sufficient Zn (1 $\mu$ M) and high Zn (10 and 50  $\mu$ M Zn for *T. arvense* and *T. caerulescens*, respectively) in roots and shoots.



**Fig. 2. Expression of the *T. caerulescens* transcription factors, *TcE2F1* and *TcE2F2*, in the yeast mutant  $\Delta zap1$  restored growth at low Zn.**

## CONCLUSIONS

With a number of genes that encode metal transporters and possible metal ligands and show elevated expression in *T. caerulescens*, it may be possible that there is a common molecular basis for hyperexpression, leading to the hyperaccumulation phenotype.

Activation of ZRT1 by *TcE2F1* and *TcE2F2* is also supportive evidence that these transcription factors play a role in regulation of Zn-transporter gene expression.

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