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Title

The Molecular Basis for Zn Hyperaccumulation in *Thlaspi caerulescens*

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Introduction

Thlaspi caerulescens (J&C Presl) is a Zn/Cd hyperaccumulator which 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). A number of different Zn transport processes in the plant 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 were all shown to exhibit 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 *T. caerulescens* possesses, as another Cd hyperaccumulator, *Arabidopsis halleri*, has also been shown to exhibit hyperexpression of multiple genes involved with micronutrient/heavy metal homeostasis (Becher et al, 2004; Weber et al 2004). One of the goals of this research is to identify factors involved in these changes in gene expression for Zn (and other micronutrient) transporters in hyperaccumulating plant species. From this research, 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 expression of specific plant Zn transporter genes. As seen in Figure 1, both of these transcription factors are hyperexpressed in *T. caerulescens*. Furthermore, both transcription factors, when expressed in yeast, can replace the function of *ZAP1*, a yeast transcription factor that helps mediate the Zn-dependent regulation of expression of a number of yeast Zn transporters that are closely related to the *T. caerulescens* Zn transporter gene, *ZNT1*.

Results and Discussion:

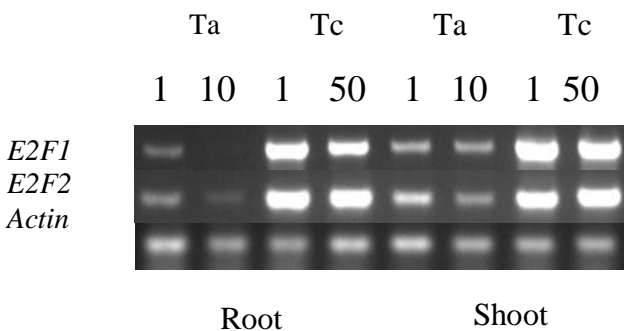


Figure 1: Expression of E2F1 and E2F2 in both *T. arvense* and *T. caerulescens* under sufficient Zn (1 μM) and high Zn (10 μM Zn for *T. arvense* and 50 μM Zn for *T. caerulescens*) in both roots and shoots.

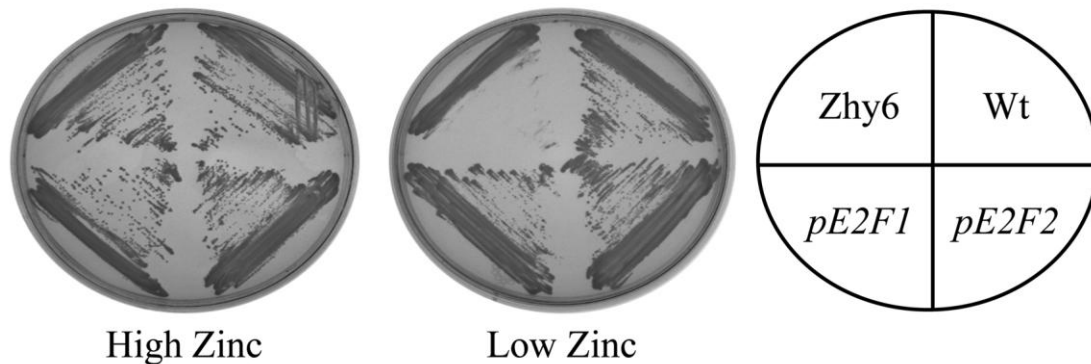


Figure 2: Expression of the *T. caerulescens* transcription factors, *TcE2F1* and *TcE2F2*, in the yeast mutant $\Delta zap1$, restores the ability of the yeast to grow on low Zn.

Conclusions:

- With a number of genes encoding metal transporters and possible metal ligands showing elevated expression in *T. caerulescens*, it may be possible that there is a common molecular basis for hyperexpression, leading to the hyperaccumulation phenotype.
- Restored growth of the $\Delta zap1$ mutant by *TcE2F1* and *TcE2F2* also is supportive evidence that these transcription factors do play a role in regulation of Zn transporter gene expression.

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