Zinc Accumulation and Tolerance Traits in *Thlaspi caerulescens* Suspension Cells

Melinda Klein[1], Matthew Milner[2], Hitoshi Sekimoto[3], Randy Clark[2], Ashot Papoyan[2], Leon Kochian[2]

[1] Department of Pediatrics, Baylor College of Medicine, USDA-ARS Children's Nutrition Research Center, Houston, TX, USA
[2] Department of Plant Biology, Cornell University, USA
[3] Department of Plant Science, Faculty of Agriculture, Utsunomiya University, JAPAN

INTRODUCTION

The metal hyperaccumulator *Thlaspi caerulescens* (J&C Presl) can accumulate Zn up to 3% of its dry weight in the above ground tissue without any negative effects on photosynthetic processes (Kochian et al. 2002 and references therein). The ability of metal hyperaccumulating plant species to accumulate such high concentrations of metals requires the coordination of uptake, transport and sequestration processes to avoid the effects seen in non-hyperaccumulating plants treated at similar metal concentrations. Previous studies of *T. caerulescens* examined metal tolerance and accumulation behaviour of hyperaccumulating plants using whole plant, organ and protoplast specific studies (Frey et al. 2000, Küpper et al. 1999, Lasat et al. 1996, 1998, Ma et al. 2005). Suspension cell based studies, to date, have not been completed with *T. caerulescens*. By producing *T. caerulescens* suspension cell lines, a system would become available that allows longer-term cellular level studies of hyperaccumulation without the perturbation of protoplast production or the artificial selection of high metals on suspension cells from non-hyperaccumulating species. With these benefits in mind, stable *T. caerulescens* suspension cell lines were created. The subsequent physiological and molecular characterization of these lines and the stable *Agrobacterium* mediated transformation of these suspension cell lines are described.

METHODS

Suspension cell lines were created in a two-step process with *T. caerulescens* (Prayon) seedlings grown on MS media under callus promoting conditions. Calli produced were transferred to liquid culture (modified MS media), and lines with consistent growth were used in characterization studies. Suspension cell growth was compared over time between *T. caerulescens* lines produced and *Arabidopsis thaliana* suspension cell lines. Subsequent studies examined biomass production and cellular Zn and Cd concentrations when cells were grown over a range of Zn and Cd concentrations. The molecular activities of these suspension cell lines were examined with semi-quantitative Polymerase Chain Reaction analysis (RT-PCR) for a number of genes previously implicated in Zn and Cd hyperaccumulation. Finally, the suitability of these cell lines for *Agrobacterium tumefaciens* mediated transformation was tested with a GFP/GUS reporter construct.

RESULTS AND DISCUSSION

Suspension cell lines from *T. caerulescens* were successfully made. These lines grew better when provided with elevated Zn concentrations suggesting a higher cellular requirement for Zn. *T. caerulescens* suspension cells did not show significant growth reduction when treated at the highest Zn concentrations tested (1.5mM Zn) while *Arabidopsis* suspension cell growth was reduced to 4% of growth relative to unsupplemented media. However, when grown at identical Zn concentrations, *T. caerulescens* cell lines accumulated lower Zn concentrations relative to *Arabidopsis*.

Molecular studies found constitutive and Zn-status dependent expression of transporters previously implicated in Zn/Cd hyperaccumulation. *T. caerulescens* cell lines showed increased expression of *HMA4*, *ZNT1* and *MTP1* relative to *A. thaliana* suspension cells. This behaviour mimicked the elevated gene expression seen at the whole plant level for *T. caerulescens* relative to related non-hyperaccumulators.

Stable transformation of the *T. caerulescens* suspension cell lines using GFP/GUS reporter constructs will be shown. Through transformation of *T. caerulescens* suspension cell lines via *Agrobacterium* and subsequent screening of cell lines under variable Zn conditions, we can better understand the molecular mechanisms at work at the cellular level leading to hyperaccumulation. Previous work leading to genetic manipulation of *T. caerulescens* has been limited by low plant fertility and long life cycle (Peer et al. 2003). The modification of gene expression in suspension cells will allow faster and more efficient screening of genes of interest within *T. caerulescens*.

ACKNOWLEDGEMENTS

We would like to acknowledge the generous gift of *Arabidopsis thaliana* suspension cells provided by the laboratory of Dr. Roger Innes (Indiana University).

REFERENCES

Cosio, C., DeSantis, L., Frey, B., Diallo, S. and Keller, C. (2005) Distribution of cadmium in leaves of Thlaspi caerulescens. J Exp Bot 56: 765-775.

Frey, B., Keller, C., Zierold, K. and Schulin, R. (2000) Distribution of Zn in functionally different leaf epidermal cells of the hyperaccumulator Thlaspi caerulescens. Plant Cell Environ 23: 675-687.

Kochian, L.V., Pence, N.S., Letham, D.L.D., Piñeros, M.A., Magalhaes, J.V., Hoekenga, O.A. and Garvin, D.F. (2002) Mechanisms of metal resistance in plants: aluminum and heavy metals. Plant Soil 247: 109-119.

Küpper, H., Jie, Z.F. and McGrath, S.P. (1999) Cellular compartmentation of zinc in leaves of the hyperaccumulator Thlaspi caerulescens. Plant Physiol 119: 305-312.

Lasat, M.M., Baker, A.J. and Kochian, L.V. (1996) Physiological characterization of root Zn^{2+} absorption and translocation to shoots in Zn hyperaccumulator and nonaccumulator species of Thlaspi. Plant Physiol 112: 1715-1722.

Lasat, M.M., Baker, A.J. and Kochian, L.V. (1998) Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in Thlaspi caerulescens. Plant Physiol 118: 875-883.

Ma, J.F., Ueno, D., Zhao, F.J. and McGrath, S.P. (2005) Subcellular localisation of Cd and Zn in the leaves of a Cd-hyperaccumulating ecotype of Thlaspi caerulescens. Planta 220: 731-736.

Peer, W.A., Mamoudian, M., Lahner, B., Reeves, R.D., Murphy, A.S. and Salt, D.E. (2003) Identifying model metal hyperaccumulating plants: germplasm analysis of 20 Brassicaceae accessions from a wide geographical area. New Phytologist 159: 421-430.