Project ID: **55118**

Project Title: **Plant Rhizosphere Effects on Metal Mobilization and Transport**

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Progress Report

RESEARCH OBJECTIVE

A mechanistic understanding of mobilization or immobolization of nutrient and pollutant metal ions by plants is largely lacking. It begins with a lack of knowledge on the chemical nature of rhizosphere components that are reactive with metal ions. This fundamental knowledge is critical to the design and implementation of phytoremediation for metal-contaminated DOE sites. Therefore, the objectives of this project include

(1) To obtain a comprehensive composition of major organic components in plant root exudates as a function of different metal ions and plant species;
(2) To examine plant metabolic response(s) to these metal ion treatments, with emphasis on production of metal reactive compounds;
(3) To investigate the effect(s) of soil microbial (e.g. mycorrhizae) association on (1) and (2).

RESEARCH PROGRESS AND IMPLICATIONS

This report summarizes work from May, 1998 through May, 1999. Although the project is scheduled to end in Sept., 1999, a no-cost extension for one year has been requested, which would allow completion of ongoing work and a more extensive investigation on a new preliminary finding (see below).

In this report period, work has been focused on examining the composition of root exudates and metabolic changes in wheat and barley plants as function of transition metal and Cd ion treatments. In addition, the gel electrophoretic method for characterizing tissue sulfhydryl group-rich peptides (important to Cd sequestration) is near completion. Experiments had the emphasis on wheat since a full set (7) of Chinese Spring (CS) genotypes are available (from Dr. J. Dvorak, UC Davis), each with one chromosome addition from the salt and Fe-deficency tolerant European saltgrass (*Lophopyrum* sp.). The exudate composition of the full-complement hybrid (AgCs) between CS and saltgrass as a function of Fe deficiency and Cd treatments has been investigated and described in the 1998 report.

The exudate profile differed significantly between barley and wheat, and also between the two wheat genotypes (Figure 1). The main difference was a Tris-like compound (X), which increased substantially in exudation in response to Cd in AgCs wheat but not in CS wheat and barley. This differential response was related to a higher Cd content in AgCs tissues, which suggest a role of compound X in Cd sequestration. This is, to our knowledge, a novel new finding. In addition, the synthetic capacity for compound X is likely to be derived from the saltgrass instead of CS wheat since it was absent in the latter exudate (Figure 1). For CS wheat, increased acetate exudation was induced by Cd treatments, which suggests its role in Cd sequestration. A partial structure of compound X is illustrated in Figure 1 based on the NMR data, and additional structure information is being acquired using high-resolution Fourier transform mass spectroscopy.

The mugineic acids (MAs) phytosiderophores (PS) increased in exudation under Fe deficiency in barley (3-*epi*-hydroxy-MA, MA, and 2'-DMA) and wheat genotypes (2'-deoxymugineic acid, 2'-DMA), which is consistent with their role in Fe acquisition. In addition, the MAs may be involved in the sequestration of Cu, Zn, and Mn. However, the MAs were not important to Cd acquisition by barley and wheat plants since their exudation was attenuated by Cd treatments. This is contrary to the prevailing notion that PS may be generally involved in heavy metal uptake by plants. The emerging evidence points to the involvement of different metal reactive compounds (MRC) in the mobilization of di- and tri-valent metal ions in the rhizosphere.

In addition to the MRC exuded by plant roots, the role of soil humates (an important class of MRC in the rhizosphere) in metal ion sequstration and root exudation was explored. This information is grossly lacking but necessary for understanding metal ion transport and mobilization in the real world. The analysis to date indicates that humates enhanced sequestration of Mn, Cu, Ni, Zn, and Cd by wheat plants. This effect was contrary to the expected role of humates as a competitive chelator for metals ions. Whether this effect is mediated through root exudation or other metabolic changes is being examined. Moreover, 7 wheat CS genotypes (each with one saltgrass chromosome addition) were characterized for their capacity for metal ion sequestration and root exudation profile. A preliminary analysis indicates that at least two genotypes exhibited significantly different sequestration profile from the CS parent, and that the sequestration profile of Cd differed from those of the transition metals. It is likely that the metal ion sequestration system acquired from the saltgrass may reside in more than two chromosomes.

The MRC involved in intracellular sequestration of metal ions was investigated using the newly developed method that combined fluorescent tagging of SH-rich peptides by bromobimane with SDS-PAGE. This method allowed a fast and simultaneous assay of both phytochelatins (PC) and metallothionein-like proteins, as illustrated for wheat, rice, and clam in Figure 2. Existing methods for PC can take over 90 min per sample of instrument run time, while the SDS-PAGE method has the same sample preparation and can perform up to 40 samples per hour.

For both wheat and rice, Cd treatments induced a large production of <3.5 kD SH-rich peptides in both root and shoot tissues. These peptides were found to belong to the family of PC since they exhibited the characteristic amino acid composition of PC. They appeared to be specifically involved in the intracellular sequestration of Cd since their production was highly correlated with the Cd content instead of the transition metal content in the tissue.

A number of non-peptidic tissue MRC (e.g. >35 amino acids, organic acids, and MAs) in wheat were also investigated using GC-MS and NMR. No significant increase (if not decrease) in their production was observed, which is consistent with a major role of PC in intracellular Cd sequestration. In addition, Cd treatments led to a dramatic reduction in amino acid content, particular for Gn and Asn, which suggests that Cd inhibits N assimilation and/or increases utilization of amino acids. This metabolic symptom may underlie the growth reduction effect of Cd. Moreover, Cd appeared to partially reverse the chlorotic effect of Fe deficiency by maintaining a higher level of chlorophylls.

PLANNED ACTIVITIES

A one-year no-cost extension has been requested to allow further characterization of compound X and investigation of the unexpected role of soil humates on metal ion sequestration by plants. For identifying the structure of compound X, we expect that molecular formula determination from high-resolution MS should be sufficient in conjunction with the available NMR data. If not, HPLC purification of compound X will be performed, followed by determination of elemental composition. Metal binding property of the purified compound X will also be determined. If time permits, we will determine which chromosome(s) of wheat genotypes govern the exudation of compound X.

Additional plant growth experiments will be conducted to study the effect of different soil humates on metal ion sequestration, root exudation, and metabolic changes in wheat. Under a separate EPA-funded project, detailed structures of several humates have been acquired and they exhibited a pronounced difference in metal-binding groups. Functional understanding of these humate structure features should lead to an unprecended insight into metal ion mobilization in the rhizosphere.

Assignment of the <3.5 kD gel bands in Figure 2 to specific PCs (e.g. PC_2, PC_3, PC_4, etc will be performed by relating the gel pattern of Cd-treated *Saccharomyces pombe* (obtained from Dr. D. Ow, USDA) to the established HPLC separation of different PCs. This development should assist in the molecular genetic manipulation of PCs synthesis by facilitating the analysis of the end products.

INFORMATION ACCESS

"NMR in the Plant-Soil Environment", T.W.-M. Fan and A.N. Lane, *in Encyclopedia of NMR Spectroscopy*, John Wiley and Sons, New York, under review.

"Comprehensive Analysis of Organic Ligands in Whole Root Exudates Using NMR and GC-MS", T.W.-M. Fan, J. Pedler, A.N. Lane, D. Crowley, and R.M. Higashi, *Analytical Biochemistry* 251, 57-68 (1997).

"Association of desferrioxamine with humic substances and their interaction with cadmium(II) as studied by pyrolysis gas chromatography mass spectrometry and nuclear magnetic resonance spectroscopy", R.M. Higashi, T.W.-M. Fan, A.N. Lan, *Analyst* 123(5), 911-918 (1998).

"Recent Advancement in Profiling Plant Metabolites by Multi-Nuclear and Multi-dimensional NMR", T.W.-M. Fan. In: Nuclear Magnetic Resonance in Plant Biology, Y. Shachar-Hill and P.E. Pfeffer, eds., American Society of Plant Physiologists, Rockville, MD, pp. 181-254 (1996).

"Ternary Interactions of Biogenic Ligands and Cd(II) With Humic Substances, With Implications for Metal Ion Bioavailability", Richard Higashi, Teresa Fan, Fabienne Baraud, and Andrew Lane, *abstr for* 1999 Semi-annual meeting of the American Chemical Society, Anaheim, CA (Mar, 1999).

"Rhizosphere Mobilization Of Heavy Metals Via Plant Root Exudation", Teresa W.-M. Fan, Moshe Shenker, Richard M. Higashi, David, E. Crowley, and Andrew N. Lane, *abstr for* Semi-Annual Meeting of American Chemical Society, Anaheim (Mar, 1999).

"Plant Rhizosphere Effects on Metal Mobilization and Transport", Teresa W.-M. Fan, Richard M. Higashi, and David E. Crowley, *abstr for* DOE EMSP Symposium, Chicago (July, 1998).

"Ternary Interactions Of Cd(II), Ligands, And Humic Substances - Implications For Metal Ion Bioavailability", Richard M. Higashi and Teresa W.-M. Fan, *abstr for* EPA, DOE, ONR, & NSF Joint Workshop (May, 1998).

"Comprehensive Determination of Root Exudates Under Combined Fe deficiency/Cd Stress by NMR and GC-MS", T.W.-M. Fan, M. Shenker, A.N. Lane, D. Crowley, and R.M. Higashi, *abstr for* Society of Environmental Toxicology and Chemistry-Europe, Bordeaux, France (April, 1998).

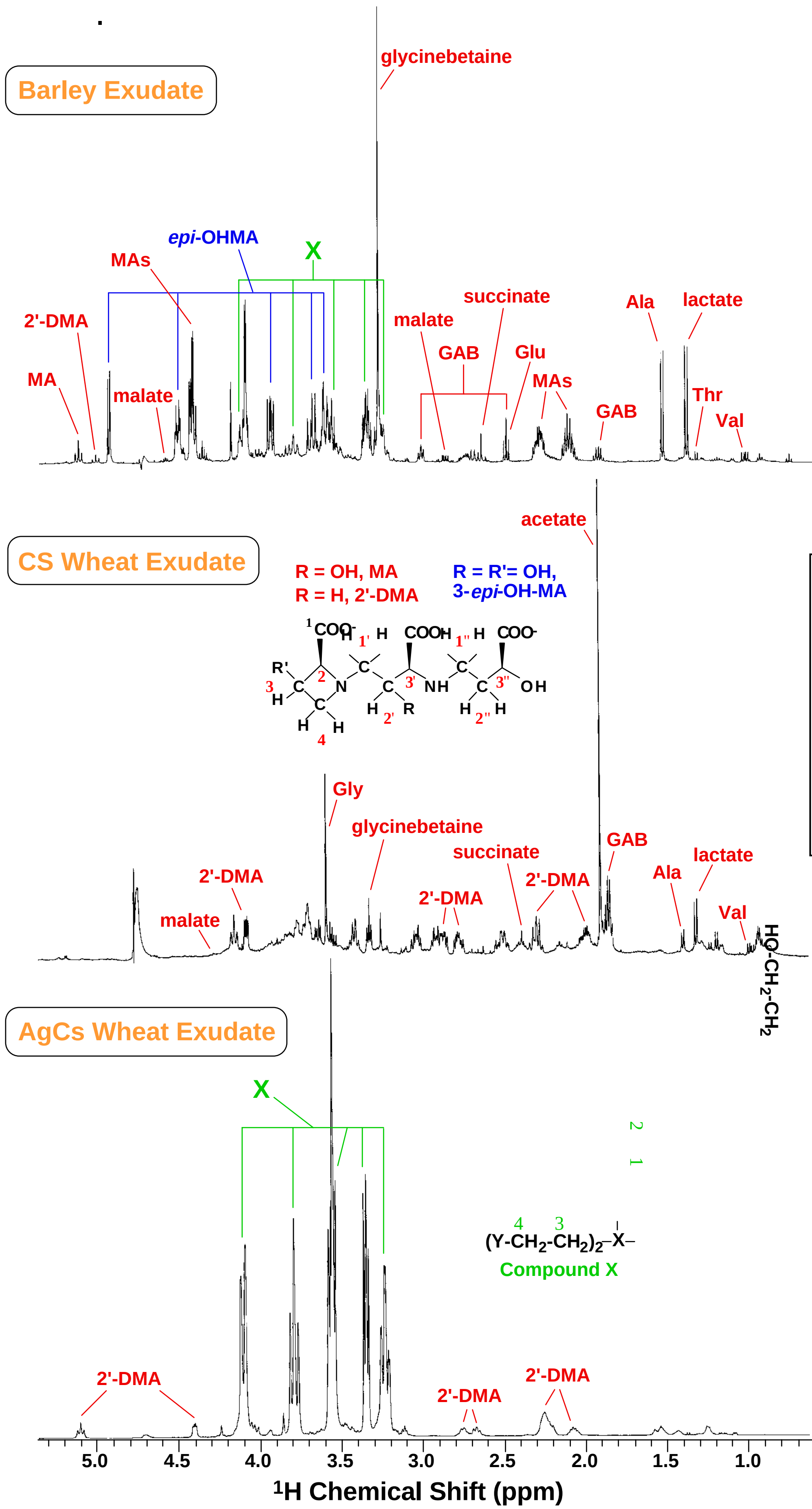

Figure 1. ^{1}H NMR spectra of whole root exudates from barley and two wheat genotypes (Chinese Spring and the hybrid AgCs) under a combined Fe deficient and Cd treatment.

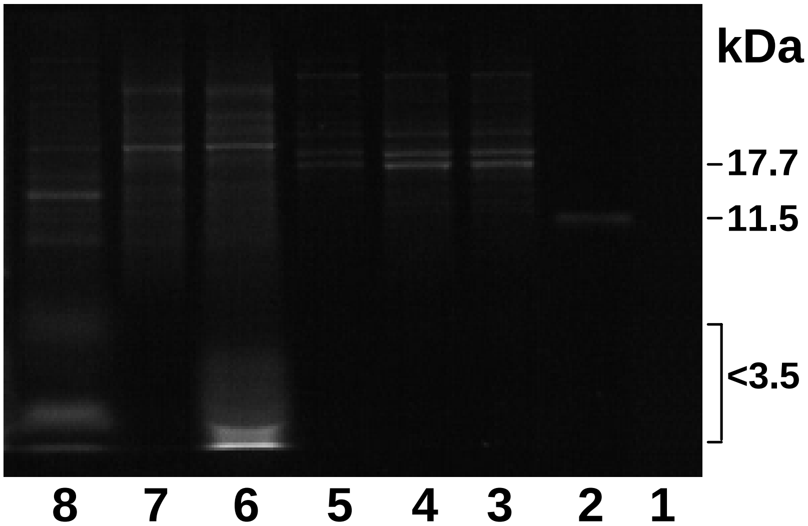

Figure 2. SDS-PAGE of bromobimane-tagged SH-rich peptides and proteins from wheat, rice, and clam tissues. Lanes 1, MW standards; 2, rabbit metallothionein (MT); 3-5, clam MT extracts; 6,7, Cd-treated and control wheat root extracts; 8, Cd-treated rice shoots, respectively.