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Report:

Manganese (Mn) is an essential element necessary for activation of a wide range of enzymes in the cell for every organism. To fulfil its metabolic functions Mn is only required at low concentrations. In general, excess Mn causes disorder of normal metabolism. While essential biologic functions of Mn depend on its oxidation state (e.g., Mn^{2+} and Mn^{3+}), little is known about how the oxidation state of elevated Mn exposures affect cellular uptake, and function/toxicity. The different properties of Mn^{2+} and Mn^{3+} and their interaction during oxidative events may be particularly relevant in the context of biological systems. Although the divalent state is the most stable form of manganese in aqueous solutions, the trivalent state is the dominant state of manganese in many biological tissues and in natural water sources (Reed, 1986). In animals, the data of Reaney et al., 2006, substantiate the heightened susceptibility of the part of the brain to manganese, and they indicate that the oxidation state of manganese exposure may be an important determinant of tissue neurotoxicity. Recently, it was shown that the valency of manganese ions, present Mn^{3+} even in trace amounts, might extensively modify the capacity of manganese to generate free radicals (HaMai and Bondy, 2004). In plants, Mn^{2+} is readily oxidized by a peroxidase system of the cell walls, thus accelerating the reactions leading to tissue injury by modifying membrane functions and oxidizing organic constituents (Horst, 1988). It has been proposed that the apoplast of plants, the compartment between cell wall and plasma membrane, plays an important role in the defence against Mn toxicity (Horst et al., 1999). Archibald and Fridovich (1982) showed that Mn^{2+} could first oxidized by $O_2\bullet^-$ to Mn^{3+} and H_2O_2 is formed. This reaction is supposed to happen on plant cell walls (Kono et al, 1976). Oxidation of Mn^{2+} by H_2O_2 consuming enzyme peroxidase has been proposed to be a key reaction leading to manganese toxicity symptoms, probably via the formation of reactive intermediate compounds such as phenoxy radicals and Mn^{3+} (Horst, 1988).

If Mn^{3+}, the first oxidation product of Mn^{2+}, is not stabilized by complexation, it can act as a powerful oxidant of numerous organic molecules, leading to toxic effects.

$$Mn^{2+} + O_2 \rightarrow [Mn^{2+}\text{-}O_2 \rightarrow Mn^{3+}\text{-}O_2^{\bullet-} \rightarrow Mn^{3+} + O_2\bullet$$

It was supposed that the pro-oxidant activity associated with divalent Mn depends on the trace presence of trivalent metal ion, most probably Mn^{3+} (HaMai et al., 2001).

Our earlier results demonstrated variable sensitivity to manganese toxicity, associated with differing transport and translocation properties in different compartments of the plant cells, in two varieties of Douglas fir (Dučić and Polle, 2007). More resistant variety show retention in root system, and on the cellular level, Mn toxicity caused the appearance of "black bodies", containing extremely high Mn concentrations, but it is not know in which oxidative state.

Results

The scanning transmission X-ray microscope (STXM) at ID21 with new cryo-stage is one of few instruments worldwide that allows for XANES-studies at the K-absorption edge of manganese at E = 6539 eV combined with high spatial resolution. Using this instrument gives the unique opportunity to examine Mn in plant cells with both, high spatial and high spectral resolution and by measurements under cryo- conditions without changing the oxidative state.

In our study, firstly we visualized Mn in plant cells and determinate the oxidation state of Mn against the spatial distribution. For this, first of all images and spatially resolved spectra were obtained of 8µm thin cryo cross-sections of root from differently treated plants. The fluorescence detector was used to obtain maps of the Mn distribution within these cells. These maps were leading us to Mn rich areas. NEXAFS-spectra were taken to identify the oxidation state at these spots. We were taking images at the peak energies of the respective resonance lines from Mn.

Cryo cross-sections of plant samples were transported under liquid nitrogen and mounted on the cryo-stage at ID21 under liquid nitrogen to have pristine objects as close as possible to a natural state.

In this study we explored first total Mn distribution within the cells (Fig. 1A) and find the redox state of manganese in the whole area of cross section of different plant tissues of root of 2-months young Douglas fir (Fig 1B, C). We were especially focused in Mn- oxidative state in different compartments of the root cells. The deposition and sequestration on Mn in an oxidative form may constitute a possible mechanism of detoxification. Especial emphasizes have been given to the processes in the apoplast in different cell types. X-ray spectromicroscopy brought a "view" in this first compartment of plant, which is in contact to the external environment and thus the first to deal with Mn toxicity and Mn oxido-redox processes. Our finally goal is to find out which kind of chemical reaction happens in the apoplast, what possibly will help to understand plants behaviour under Mn excess.

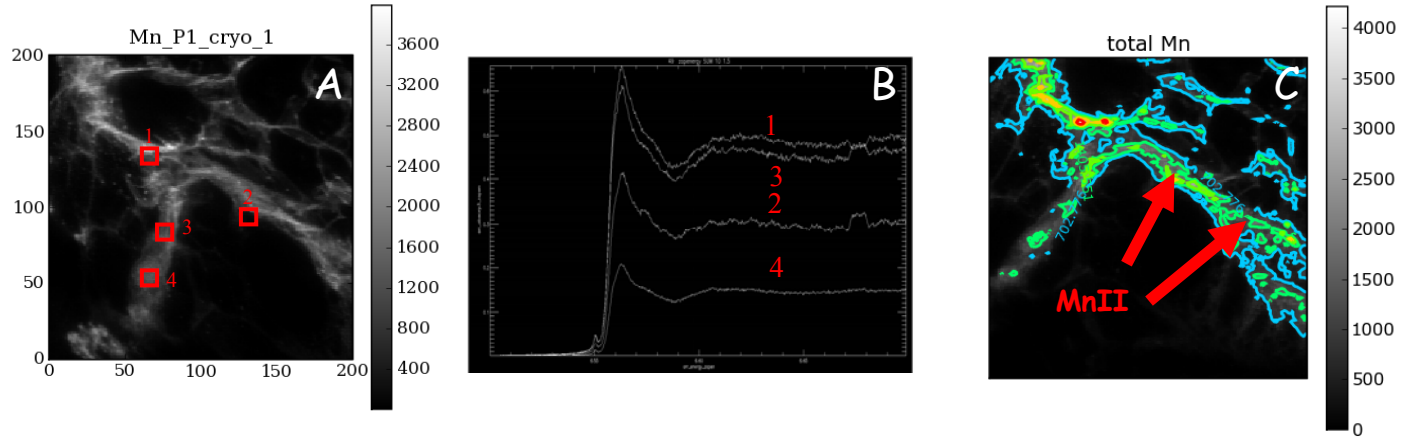

Fig. 1. Total Mn mapping in endodermis cells of Douglas fir root (A). Spots 1-4 were analyzed by using XANES (B). X-ray scanning microscopy at two different energy levels (6.56keV and 6. 7 keV) shows Mn^{3+} deposition in the cells (C).

References

Archibald FS, Fridovich I. 1982. *Archive of Biochemistry and Biophysics* **214:** 452–463.

Dučić T, Polle A. 2007. *Functional Plant Biology*. **34**: 31-40

HaMai D, Bondy SC 2004. *Annals of the New York Academy of Sciences* **1012:** 129–141.

HaMai D, Campbell A, Bondy SC 2001. *Free Radical Biology and Medicine* **31**:763-768.

Horst WJ, Fecht M, Naumann A, Wissemeier AH, Maier P. 1999. *Journal of P Nutrition and Soil Science* **162:** 263-274.

Horst WJ. 1988. *Manganese in Soils and Plants*. Kluwer Academic Publishers, Nederland 175–188.

Kono Y, Takahashi MA, Asada K. 1976. *Archive of Biochemistry and Biophysics* **174:** 454–462.

Reaney SH, Bench G, **Smith DR 2006.** *Toxicological Sciences* **93**:114-124.

Reed GH. 1986. *Manganese in metabolism and enzyme function*, Academic Press, New York pp. 313–323.