Characterization of Mn-induced apoplastic peroxidases in cowpea (*Vigna unguiculata* L.)

Stefanie Götze[1], Hendrik Führs[1*], Hans-Peter Braun[2], Walter Johannes Horst[1]

[1] *Institute of Plant Nutrition, Faculty of Natural Sciences, Leibniz University Hannover, Herrenhäuser Str. 2, 30419 Hannover*
[2] *Institute of Plant Genetics, Department of Plant Molecular Biology, Faculty of Natural Sciences, Leibniz University Hannover, Herrenhäuser Str. 2, 30419 Hannover*
[*] *Corresponding author: Hendrik Führs hendrik.fuehrs@pflern.uni-hannover.de*

Introduction: Apoplastic H_2O_2-producing peroxidase (POD, NADH-peroxidase) and H_2O_2-consuming POD (guaiacol-POD) are proposed to play key roles in Mn toxicity and Mn tolerance (Fecht-Christoffers et al., 2003). Additionally, apoplastic metabolites significantly affect PODs activities (Fecht Christoffers et al., 2006). In order to characterize the Mn-induced POD isoforms, we separated proteins using blue-native polyacrylamide gel electrophoresis (BN-PAGE). After elution of specific POD isoforms we determined their pH optimum, substrate specifity, and inhibitory effects of different phenols and organic acids.

Results:

1. Several POD isoforms were induced by elevated Mn supply

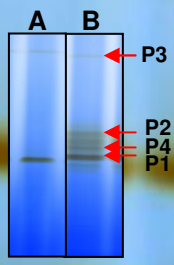

*Fig.1. BN-PAGE of apoplastic proteins of the Mn-sensitive cv TVu 91. Plants received either 0.2 µM Mn (**A**) or were treated with 50 µM Mn for 4 d (**B**). Marked isoforms were used for further characterization.*

Materials and Methods: Apoplastic Washing Fluid (AWF) was extracted using a vacuum-infiltration/centrifugation technique (Fecht-Christoffers et al., 2003). Proteins were separated from AWF into protein concentrate and metabolite filtrate using centrifugal concentrators and then separated by BN-PAGE (Fecht-Christoffers et al., 2003). Proteins of interest were eluted from the gels (Werhahn und Braun, 2002) and peroxidase isoform activites were determined spectrophotometrically. Measuring solution consisted of the examined phenol or a combination of phenols, $MnCl_2$, NADH and sample.

2. Almost each isoform catalysed both, guaiacol-POD and NADH-*per*oxidase activity. The reaction-dependent pH optima for all isoforms were 6.5 and 5.5, respectively. One isoform (P3) hardly had guaiacol-POD activity.

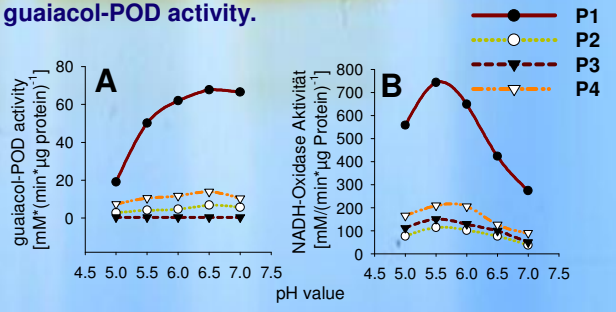

*Fig.2. Effect of the pH on the guaiacol-POD (**A**) and NADH-peroxidase (**B**) activity of different POD isoforms.*

3. Only four of nine phenols in varying concentrations affected NADH-*per*oxidase activity of the examined POD isoforms, but each of them in a different way.

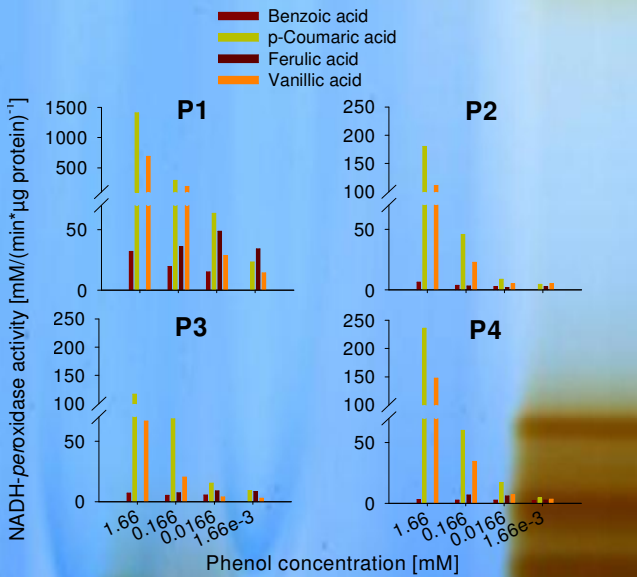

Fig.3. Effect of different phenols and their concentrations on the NADH-peroxidase activity of different POD isoforms.

4. Combining *p*-coumaric acid with different phenols mostly reduced max. NADH-*per*oxidase activity. Only benzoic acid and vanillic acid enhanced it.

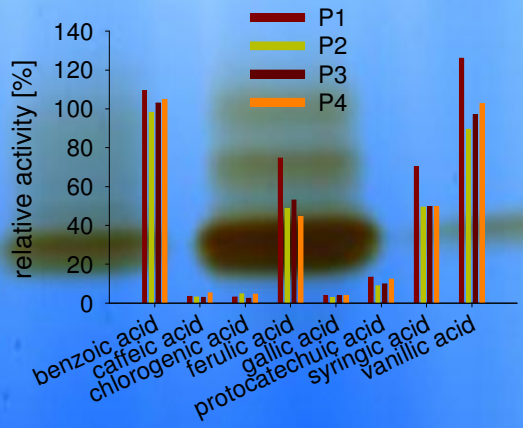

Fig.4. Effect of the different phenols on max. NADH-peroxidase activity of different POD isoforms. Plots are displayed as relative values to the activity only with p-coumaric acid.

Conclusions: The fact, that different phenols exerted differential enhancing and inhibitory effects on NADH-*per*oxidase activities confirm the hypothesis that not only PODs but also apoplastic phenolic metabolites are key factors in developing Mn toxicity and Mn tolerance.

References:

Fecht-Christoffers M. M., Braun, H.-P., Lemaitre-Guillier C., VanDorsselaer A., Horst W. J., 2003, Plant Physiology, 133, 1935-1946

Fecht-Christoffers M.M., Führs H., Braun H.-P., Horst W.J., 2006, Plant Physiology, 140, 1451-1463

Wehrhahn W and Braun H.-P., 2002 , Electrophoresis, 23, 640-646

This work was supported by the German Research Foundation (DFG) through Ho 931/17-1