The role of phenols in the modulation of apoplastic peroxidases and their role in Mn toxicity and Mn tolerance in cowpea (*Vigna unguiculata* L.)

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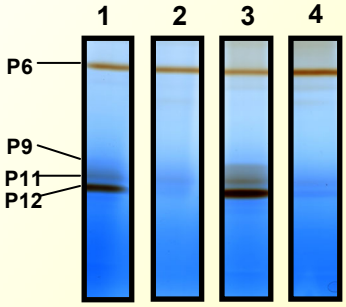
Introduction: Manganese and apoplastic metabolites, e.g. phenylpropanoids, significantly affect peroxidases, especially NADH-*per*oxidase activity (Fecht Christoffers et al., 2006) which is regarded as a key reaction in the development of Mn toxicity. In order to characterize peroxidase isoenzymes, we separated apoplastic proteins of two cowpea cultivars differing in Mn tolerance (TVu 91, Mn-sensitive; TVu 1987, Mn-tolerant) using Blue-Native polyacrylamide gel electrophoresis (BN-PAGE). After elution of specific POD isoenzymes of TVu 91 we determined their pH optimum, substrate specifcity, and inhibitory effects of different phenols. By using a metabolite profiling approach, the non-polar apoplastic metabolite fraction of both cultivars was investigated as well.

M&M: Apoplastic Washing Fluid (AWF) was extracted using a vacuum-infiltration/centrifugation technique (Fecht-Christoffers et al., 2003). Proteins were concentrated from AWF 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 isoenzyme activites were determined spectrophotometrically. Measuring solution consisted of the examined phenol or a combination of phenols, $MnCl_2$, NADH and sample. Apoplastic non-polar phenolic compounds were extracted by shaking with diethylether after alkalinization and subsequent acidification of the AWF. Non-polar metabolites were analyzed by GC-MS based metabolite profiling (Sanchez et al., 2008, Steinfath et al., 2008).

1. POD isoenzyme patterning differed between the cultivars and Mn treatments.



1 TVu 91 -Mn
2 TVu 1987 -Mn
3 TVu 91 +Mn
4 TVu 1987 +Mn

Fig.1. BN-PAGE of apoplastic proteins stained for guaiacol-POD activity of the Mn-sensitive cv TVu 91 (1, 3) and the Mn-tolerant cv TVu 1987 (2, 4). Plants received either 0.2 µM Mn (1, 2) or were treated with 50 µM Mn for 3 d (3, 4). Marked isoenzymes were used for further characterization.

2. Almost each isoenzyme catalysed both, guaiacol-POD and NADH-*per*oxidase activity. The reaction-dependent pH optima for all isoenzymes were 6.5 and 5.5, respectively.

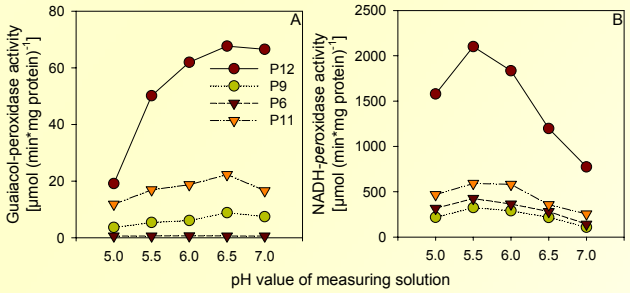


Fig.2. Effect of the pH on the guaiacol-POD (**A**) and NADH-*per*oxidase (**B**) activity of different POD isoenzymes.

3. Only four of nine phenols in varying concentrations affected NADH-*per*oxidase isoenzymes, but each in a different way.

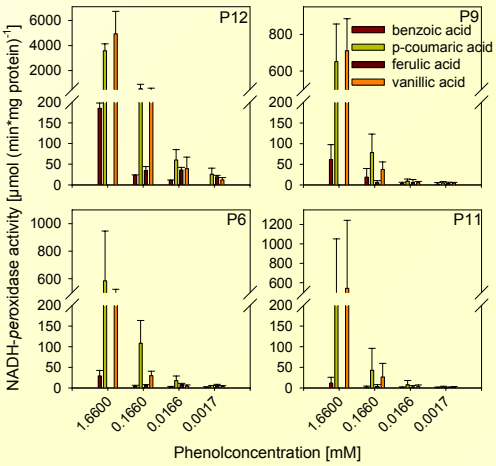


Fig.3. Effect of different phenols and their concentrations on the NADH-*per*oxidase activity of different POD isoenzymes.

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

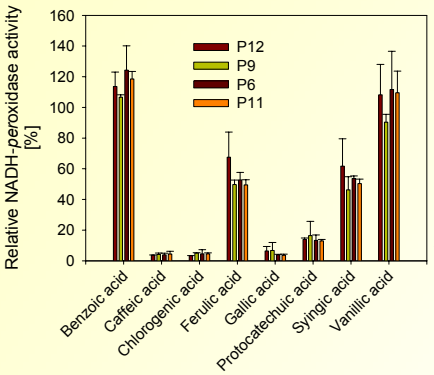


Fig.4. Interaction effects of eight different phenols with *p*-coumaric acid on max. NADH-*per*oxidase activity of different POD isoenzymes. Plots are displayed as relative values to the activity only with p-coumaric acid.

Table I: Identified phenols in the non-polar leaf AWF fraction and their relative changes in abundance (based response ratios). ***, **,* indicate significant changes in metabolite abundance at $p < 0.001$, 0.01 and 0.05, respectively (t test, n=6).

Detected phenol	TVu 1987 +Mn/-Mn	TVu 91 +Mn/-Mn
cis-p-coumaric acid	0.079**	1.06
cis-ferulic acid	only –Mn	0.27**
trans-p-coumaric acid	0.048**	0.94
trans-ferulic acid	2.58	0.44*

5. NADH-*per*oxidase inhibiting phenols are downregulated in the Mn-sensitive cv. TVu 91 due to elevated Mn-supply therefore increasing the relative importance of enhancing phenols and *vice versa* in the Mn-tolerant cultivar TVu 1987.

Conclusions: Different phenols exerted differential enhancing and inhibitory effects on NADH-*per*oxidase activities *in vitro* thus confirming the decisive role of apoplastic phenols in mediating apoplastic POD activities. Mn-mediated changes in apoplastic phenol concentrations revealed a kind of passive Mn response since (depending on the genotype) NADH-*per*oxidase inhibiting/enhancing phenols are downregulated thus leading to an increased relative importance of enhancing/inhibting phenols in terms of NADH-*per*oxidase activation. Additionally, local apoplastic pH changes might lead to a preference for either H_2O_2-consuming or H_2O_2-producing POD activity.

References: Wehrhahn and Braun H-P (2002): Electrophoresis, 23, 640-646; **Fecht-Christoffers** et al. (2003): Plant Phys. 133, 1935-1946; **Fecht-Christoffers** et al. (2006): Plant Phys. 140, 1451-1463; **Sachez et al.** (2008): Phys. Plant., 132, 209-219; **Steinfath et al.** (2008): Phys. Plant., 132, 150-161

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