Peroxidase activity in the leaf apoplast is a sensitive marker for Mn toxicity and Mn tolerance in *Vigna unguiculata*

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Introduction

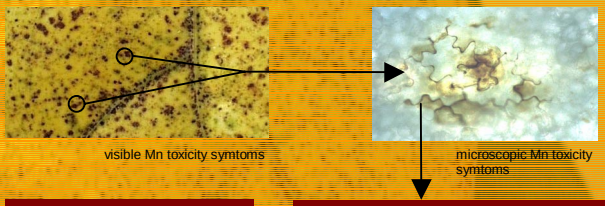
In *Vigna unguiculata* (L.) Walp. resistance to Mn excess is due to the ability of the genotypes to tolerate high Mn tissue contents in their leaves. First symptoms of Mn toxicity in older leaves are brown spots consisting of oxidized Mn and oxidized phenols in the cell wall. The oxidation of phenols by a H2O2-peroxidase (POD) system in the apoplast **is proposed** to be the key reaction leading to Mn toxicity mediated by the formation of highly toxic Mn^{III} and phenoxy radicals finally resulting in the formation of the Mn toxicity symptoms (Horst et al., 1999). **Since Mn tissue tolerance is negatively linked to the formation of these brown spots we tested the hypothesis, that Mn tolerance is due to a lower activity of POD-isoenzymes especially in the leaf apoplast.**

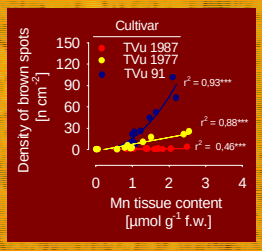
Material and methods

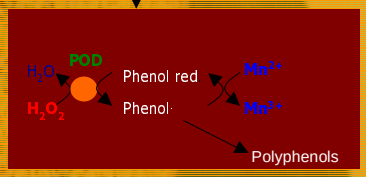
Cowpea (*Vigna unguiculata* (L.) Walp.) cultivars TVu 91, TVu 1977, and TVu 1987 were grown hydroponically under controlled environmental conditions in a growth chamber. Plants precultured for 15 days at 0.2 µM Mn were treated with 50 and 100 µM Mn, whereas control plants received 0.2 µM Mn continuously. After 5 days of Mn treatment the second oldest leaves were used for quantification of Mn toxicity symptoms and for determination of **POD activity**. Leaves were vacuum infiltrated (35 hPa) with dest. water and subsequent centrifuged (extraction of AWF) (a), homogenised, centrifuged and dialysed (b), pellets were washed and finally, cell walls were incubated with salt solutions (NaCl/LiCl) (c) followed by a pectolyase-cellulase-BSA mixture (d). Activity of **apoplastic water soluble POD** (a), **ionically-bound cell wall POD** (c), **covalently-bound cell wall POD** (d), and **cytoplasmic POD** (b) was determined spectrophotometrically by following H_2O_2-depending oxidation of guaiacol. Proteins were quantified by fluorescence. After concentrating the AWF, POD isoenzymes were separated by discelectrophoresis (native page) and stained with a guaiacol-H_2O_2 test mixture. Mn contents of leaves were detected by ICP-OES.

Mn toxicity and tolerance

symptoms and related POD catalyzed reactions in the leaf apoplast

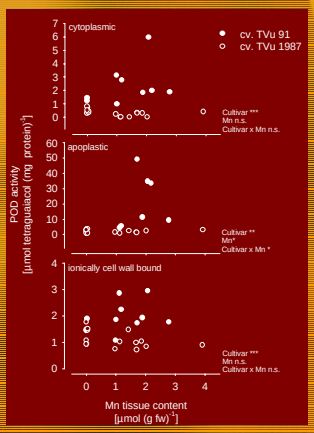






Results

Increased activity of POD in Mn-sensitive but not in Mn-tolerant leaf tissue with increasing Mn supply

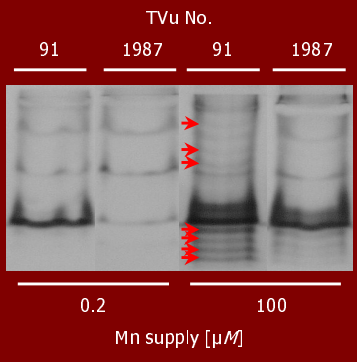


Substantial increase of the activity of the apoplastic water-soluble POD in Mn-sensitive leaf tissue with increasing Mn supply

cv. TVu 91

	Peroxidase activity [µmol tetraguaiacol (mg protein)⁻¹]			Peroxidase activity [µmol tetraguaiacol (g fw)⁻¹]		
Mn supply [µM]	0,2	100		0,2	100	
apoplastic water soluble	4,81	34,53	*	1,48	13,28	*
apoplastic ionically bound	1,64	2,15	n.s.	2,54	3,37	n.s.
apoplastic covalently bound	n.d.	n.d.		0,1	0,19	n.s.
cytoplasmatic	1,12	3,92	n.s.	3,08	6,09	n.s.

Increased quantity and intensity of POD-isoenzyme bands in the AWF with increasing Mn supply, especially in Mn-sensitive leaf tissue



Conclusion

The results clearly support the hypothesis that Mn toxicity and Mn toxicity symptoms are mediated by a H_2O_2-POD system in the leaf apoplast, since only in Mn-sensitive but not in Mn-tolerant leaf tissue especially a water soluble POD in the apoplast is activated at high Mn contents of the leaves. However, it remains unclear, whether the increase in POD activity is due to a direct stimulation of this enzyme by Mn, or indirectly via a signal pathway of an Mn-induced oxidative burst as has been proposed as a response to pathogen infection (Wojtaszek, 1997).

Literature

Horst *et al.*, 1999 J. Plant Nutr. Soil Sci. 162, 263-274.

Wojtaszek, P. 1997 Biochem. J. 322, 681-692.

This work was supported by the German Research Foundation (DFG, SSP 717) to Walter J. Horst and P. Maier