A Systems Biology Approach for the Analysis of Mn Sensitivity and Tolerance in the tropical Legume Cowpea (*Vigna unguiculata* L.)

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Introduction: Excess manganese (Mn) supply leads to enhanced apoplastic peroxidase (POD) activities (Fecht-Christoffers et al., 2006). For other plant species (González and Lynch, 1998; Houtz et al., 1988, Nable et al., 1988) changes in the photosynthetic performance have been described. Silicon supply (Si) delays Mn toxicity development in sensitive cultivars (Iwasaki et al., 2002a, b; Rogalla and Römheld, 2002). This work describes a systems biology approach applied to the apoplast and symplast to unravel mechanisms either leading to Mn toxicity development or Mn tolerance. Also, the alleviative effect of Si was investigated.

Materials & Methods: After 14 d of preculture with or without Si cowpea plants received 50 µM Mn (+Mn) for 3 d or 0.2 µM Mn (-Mn) continiously. BN-PAGE of Apoplastic Washing Fluid (AWF) proteins and subsequent guaiacol-POD activity staining was done as described in Führs et al. (2009a). IEF/SDS-PAGE and BN/SDS-PAGE was done according to Führs et al. (2008). GC-MS based metabolite profiling including non-supervised Independent Component Analysis (at http://metagenealyse.mpimp-golm.mpg.de) was carried out according to Führs et al. (2009b).

1. In the apoplast there are genotypic differences in the POD isoenzyme profile between the Mn-tolerant cowpea cultivar TVu 1987 (t) and the Mn-sensitive cultivar TVu 91 (s) (A) . In TVu 91 the isoenzyme profile is modulated by Mn and Si supply (B).

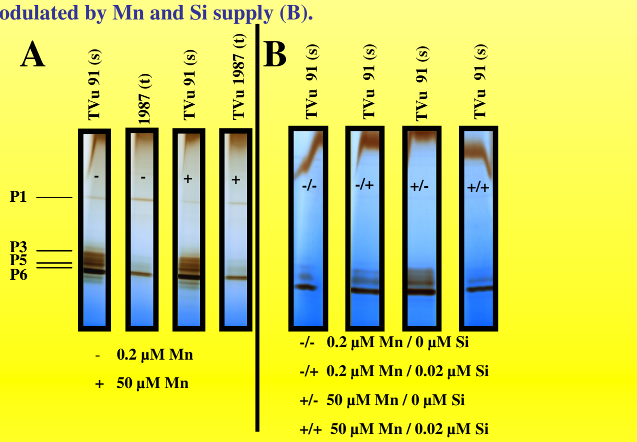

Fig. 1: (A) AWF proteins of the second oldest trifoliate leaves of the Mn-sensitive cultivar TVu 91 and the Mn-tolerant cultivar TVu 1987 and (B) AWF proteins after 0 and 4 d of Mn treatment of ±Si-treated plants of cultivar TVu 91 after separation with BN-PAGE and staining for guaiacol-POD activity.

2. In the symplast at high Mn supply, seven proteins changed in abundance in the Mn-sensitive cv. TVu 91 (s), whereas only one protein was upregulated in the Mn-tolerant cv. TVu 1987 (t).

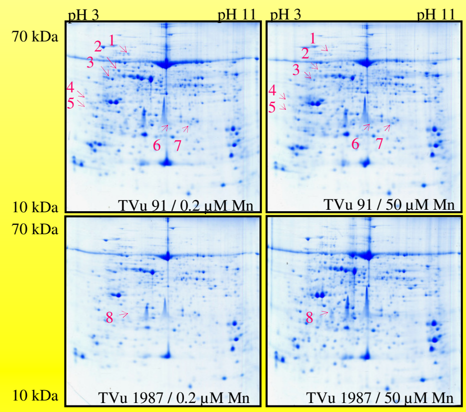

Fig.1: IEF/SDS-PAGE of the bulk-leaf proteome of two cowpea cultivars differing in Mn tolerance after 3 d of elevated (50 µM) Mn supply. Spots marked with arrows and numbers were statistically evaluated, sequenced and identified.

3. BN/SDS-PAGE revealed a state I – state II transition of photosynthesis particularly in the Mn-sensitive cultivar TVu 91 (s).

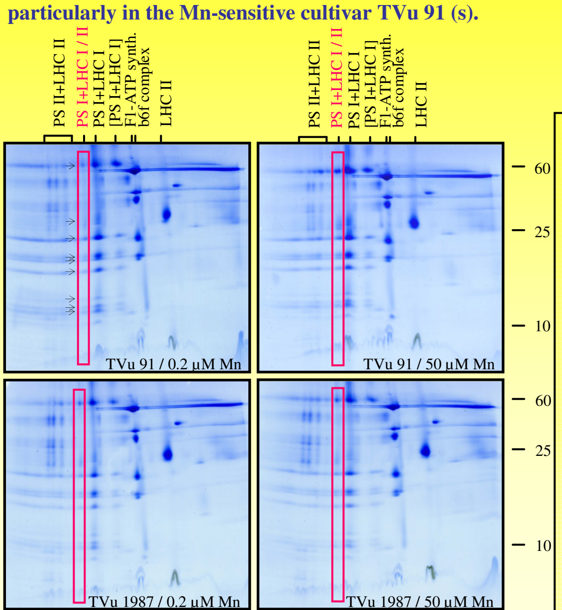

Fig.3: BN/SDS-PAGE of chloroplastic protein complexes. Arrows indicate subunits used for quantification.

References: **Fecht-Christoffers** et al., (2006): Plant Phys. 133, 1451-1963; **Führs** et al., 2008: Proteomics 8, 149-159; **Führs** et al., (2009): J. Exp. Bot 60, 1663-1678; **González and Lynch** (1998): Physiol Plant. 101, 872-880; **Houtz** et al., (1988): Plant Phys. 86, 1143-1149; **Iwasaki** et al., (2002a): J. Plant Physiol. 159, 167-173; **Iwasaki** et al., (2002b): Plant Soil 238, 281-288; **Nable** et al., (1988): Plant Phys. 86, 1163-1142; **Rogalla and Römheld** (2002): Plant Cell Environ. 25, 549-555.

4. An Independent Component Analysis (ICA) shows that the total, apoplastic and non-polar apoplastic metabolome differs between the genotypes and that it is affected by excess Mn and Si supply. The contribution of each experimental factor on metabolomic changes is quantifiable.

Conclusion: In the apoplast the higher abundance of peroxidase isoenzymes in association with a higher response of the isoenzyme profile to toxic Mn supply may explain the apoplastic Mn-sensitivity of TVu 91 compared with TVu 1987. Silicon supply is able to reduce the Mn effect on the isoenzyme profile. Also, TVu 91 responded much more on the total proteome level to toxic Mn supply than TVu 1987. Proteins of Mn-induced changed abundance mostly belonged to photosynthesis relevant processes.Indeed, also the photosynthetic protein complexes showed a swith from state I to state II photosynthesis. These state transitions induce cyclic electron transport with increased ATP production at the cost/binding of reducing equivalents (NADPH, ferredoxin). Changed photosynthesis also affects the primary and secondary metabolism in the symplast and apoplast as revealed by ICA of the metabolite profiling results. The alleviative effect of Si seems to be a constitutive effect which is then masked by excess Mn-induced processes explaining that Si only delays but not prevents Mn toxicity.

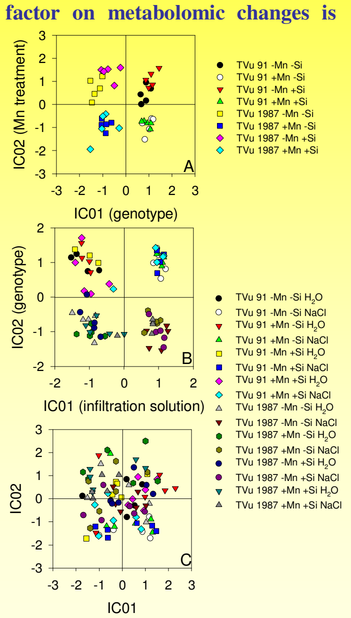

Fig. 3: ICA plot of the (A) bulk-leaf metabolites, (B) the apoplastic AWFH20 and AWFNaCl-metabolites, and (C) non-polar apoplastic metabolites extracted from the AWFH20 and AWFNaCl of the second oldest trifoliate leaves of TVu 91 and TVu 1987 as affected by Mn and Si treatments.