Transcriptomic analysis of genotypic differences in and the effect of silicon on manganese tolerance of *Vigna unguiculata* L. Walp

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Introduction

Apoplastic Mn-induced proteomic changes in the Mn-sensitive cowpea cv. TVu 91 (FECHT-CHRISTOFFERS ET AL., 2006) could be triggered by symplastic molecular events. FÜHRS ET AL. (2008) identified Mn-affected symplastic proteins in cv. TVu 91. A modified leaf-gene expression might direct these Mn-dependent differences. Thus, our aim was to investigate Mn stress-induced transcriptomic differences in two cowpea genotypes differing in Mn tolerance. Additionally, silicon (Si) was included to study early transcriptomic changes leading to Si-enhanced Mn tolerance (HORST ET AL., 1999), in the Mn-sensitive cowpea cv. TVu 91.

Mn stress induces broad range genotypic differences in the tolerant and sensitive cowpea transcriptome.

A strong constitutive and Mn-coupled Si-effect is evident in the sensitive cowpea transcriptome.

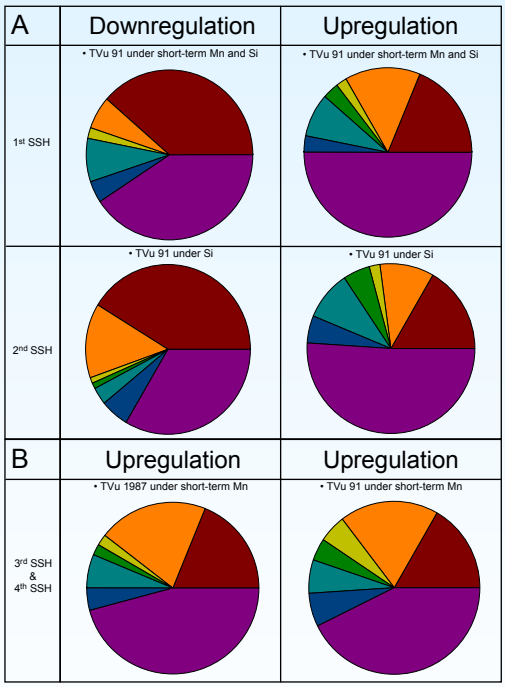

Fig 2: Differentially expressed sequences of cowpea cv. TVu 91 and cv. TVu 1987 as affected by optimum or short-term excess Mn treatment in combination +/- continuous Si supply. Sequences were categorized as described in the figure legend. (A: 1st SSH: downregulation of transcripts in TVu 91 by Si in combination with 1 day Mn and upregulation of transcripts in TVu 91 by Si in combination with 1 day Mn. 2nd SSH: Downregulation of transcripts in TVu 91 by Si and upregulation of transcripts in TVu 91 due to Si. B: 3rd SSH: Upregulation of transcripts in TVu 1987 by 1 day Mn. 4th SSH: Upregulation of transcripts in TVu 91 by 1 day Mn).

References: Fecht-Christoffers et al., 2006, Plant Physiol., 140, 1451-1463. Führs et al., 2008, Proteomics, 8, 149-159. Führs et al., 2009, J. Exp. Bot., 60, 1663-1678. Horst et al., 1999, J. Plant Nutr.Soil Sci., 162, 263-274.

We are grateful for the financial support by the DFG through HO 931/ 18-1

Materials and Methods

V. unguiculata L. Walp. cv. TVu 91 (Mn-sensitive) and cv. TVu 1987 (Mn-tolerant) were cultivated according to FÜHRS ET AL. (2008, 2009). The PCR-Select™ cDNA Subtraction Kit was used for the SSH approach. Differentially expressed sequences were cloned into the pGEM®-T Easy Vector System . Recombinant clones were analysed by custom DNA sequencing. A SYBR®Green based qRT PCR assay was designed. PCR efficiencies were analysed with a relative standard curve method. The comparative threshold cycle method ($2^{-\Delta\Delta Ct}$ method) was used for determination of the relative target quantity.

Fig. 1: Cultivation of cowpea cv. TVu 91 and cv. TVu 1987. Plants were grown hydroponically in a growth chamber at a temperature of 25/30°C, a day/night rhythm of 16/8 h, a relative humidity of 75 % ± 5 %, and a photon flux density of 150 µmol m^{-2} s^{-1} photosynthetic active radiation at mid-plant height.

Leaf apoplastic peroxidase (POD) isoenzymes show a genotype specific expression pattern in response to Mn and Si.

Long-term Mn supply increases the transcript abundance of both POD isoenzymes particularly in the sensitive cowpea cultivar.

The constitutive and Mn-coupled transcript level reducing effect of Si is POD isoenzyme dependend.

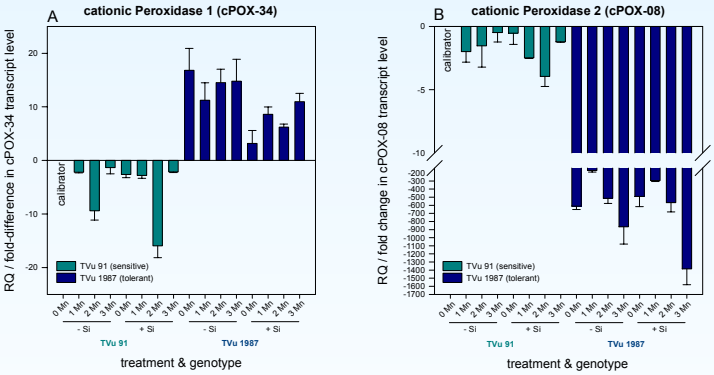

Fig. 3: Relative transcript levels of (A) cationic peroxidase 1 (cPOX-34) and (B) cationic peroxidase 2 (cPOX-08) in cowpea cv. TVu 91 and cv. TVu 1987. Data were normalised to the expression of ß-tubulin specific for *Fabaceae*, calibrated against a reference (calibrator 0Mn–Si) and expression values were equal to $1/2^{-\Delta\Delta Ct}$. Plants were pre-cultured in the presence or absence of 20 µM Si for 14 days (+/- Si) and afterwards treated with 50 µM Mn for one, two or three days (1 Mn, 2 Mn, 3 Mn), whereas control plants received 0.2 µM Mn continuously (0 Mn). Bars represent means +/- SEs of three biological replicates.

Conclusions

An excess Mn driven transcriptome modulation within both cowpea cvs. might trigger a genotype-specific apoplastic Mn-stress response. Constitutive and stress combined Si-dependent changes within the sensitive cowpea transcriptome delay an apoplastic Mn-stress reaction. These findings point to a concerted interaction of symplastic molecular events with apoplastic reactions resulting in a complex genotype-assigned Mn-stress response.

The genotype-dependent expression of polyfunctional apoplastic PODs isoenzymes could contribute to either Mn tolerance or Mn sensitivity. A reduction of POD transcripts in the tolerant genotype might cause a lower enzyme activity, possibly constraining the Mn-toxicity promoting H_2O_2-consuming POD activity. Additionally, inhibiting apoplastic phenols (FÜHRS ET AL., 2009) could counteract a transcript elevation within the tolerant genotype, thus limiting the Mn-stress enhancing H_2O_2-producing POD activity.