

Excess manganese-induced changes in the transcriptome of two cowpea (*Vigna unguiculata* [L.] Walp.) cultivars differing in manganese tolerance



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INTRODUCTION:

Silicon (Si) is known to reduce sensitivity of plants to excess Mn supply (Horst et al., 1999). Although the role of the leaf apoplast for Mn toxicity and tolerance in cowpea (*Vigna unguiculata* [L.] Walp.) is clearly established, early differences in the symplastic proteome might be directed by a Mn-induced modification of the leaf transcriptome. To identify rapid changes in the leaf gene expression which might enhance genotypic and Si-induced Mn leaf-tolerance, transcriptomic analyses were performed.

MATERIAL & METHODS:

Suppression Subtractive Hybridization (SSH) (Diatchenko et al., 1996) was used to analyse the leaf transcriptome of two cowpea cultivars differing in Mn leaf-tissue tolerance after 1-3 days of excess (50 µM) Mn treatment in combination with varying (0 and 20 µM) Si supply. The cDNA of the tester transcriptome was subtracted from the reference driver transcriptome.

RESULTS:

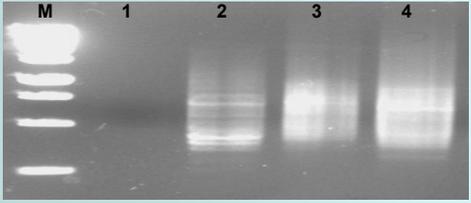


Fig.1: Selective amplification of differentially expressed transcripts from TVu 91 after 1 day of excess Mn supply in absence and presence of Si. Subtraction of **tester (Mn+Si-)** from **driver (Mn+Si+)** cDNA. 2%-Agarose-EtBr-Gel. (Lane M: marker, Lane 1: primary PCR product of subtracted tester, Lane 2: secondary PCR product of subtracted tester, Lane 3: primary PCR product of control, Lane 4: secondary PCR product of control).

The forward and reverse subtraction clearly exhibited differences in the banding pattern of the unsubtracted tester cDNA and the subtracted tester cDNA. The SSH technique significantly enriched the differentially expressed transcripts.

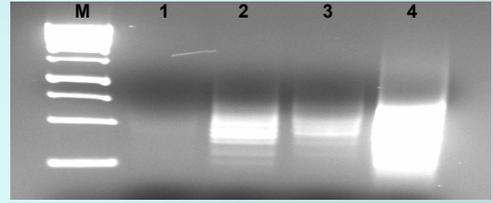


Fig.2: Selective amplification of differentially expressed transcripts from TVu 91 after 1 day of excess Mn supply in absence and presence of Si. Subtraction of **tester (Mn+Si-)** from **driver (Mn+Si+)** cDNA. 2%-Agarose-EtBr-Gel. (Lane M: marker, Lane 1: primary PCR product of subtracted tester, Lane 2: secondary PCR product of subtracted tester, Lane 3: primary PCR product of control, Lane 4: secondary PCR product of control).

Short-term excess Mn supply reduced the number of transcripts involved in photosynthesis, respiration and primary metabolism and increased the number of genes involved in signal transduction within the Mn-sensitive cultivar TVu 91. PR (pathogenesis-related) proteins were induced in both cultivars.

Transcripts from a cDNA library of the Mn-sensitive cultivar TVu 91 created 3 days after excess Mn supply showed sequence similarity to earlier identified apoplastic PR proteins (Fecht-Christoffers et al., 2003).

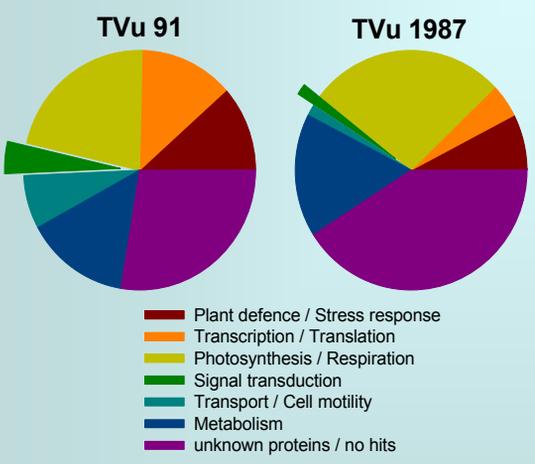


Fig.3: Short-term Mn-excess induced gene expression in the two cowpea cultivars TVu 91 and TVu 1987. The treatment of plants was 0.2 µM versus 50 µM Mn for 1 day after 14 days of pre-culture.

A) SSH clone AM748522 & AM748523, putative pathogenesis related protein 1a

...PNIVWDNT**VAAFAQNYANQR**RGDCNLIHSGGKY**YGENLAGSSGDL**SGK...

Peptide of a putative pathogenesis related apoplast protein

YGENLAGSSGDLSGK

Peptide of a putative pathogenesis related apoplast protein

VAAFAQNYANQR

B) SSH clone AM748524, putative pathogenesis related protein 4

...RAVSAYCSTYDADFA...

Peptide of a putative pathogenesis related apoplast protein

SAYCSTYDA

Fig.4: Amino acid sequence comparisons between two Mn-induced proteins of the Mn-sensitive cultivar TVu 91 identified with the SSH technique and proteins identified by proteome analyses of the apoplast proteome (Fecht-Christoffers et al. 2003).

CONCLUSIONS: The rapid up-regulation of genes involved in signal-transduction only in the Mn-sensitive cultivar indicates a role of the symplast in development of the Mn-toxicity stress-response. Short-term excess Mn treatment induced early broad-range transcriptomic differences in both cowpea cultivars confirming proteomic results. The Si-induced early genomic changes in the cowpea cultivar TVu 91 is expected to contribute to the clarification of Si-enhanced Mn-tolerance of the leaf tissue.