Title

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

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Introduction

The apoplastic processes leading to Mn toxicity in cowpea still have to be elucidated. Mn induced proteomic changes were detected within the leaf apoplast of the Mn-sensitive cowpea cv. TVu 91 (Fecht-Christoffers et al. 2006) and could be triggered by symplastic molecular events. Führs et al. (2008) were able to identify Mn-affected symplastic proteins in the Mn-sensitive cowpea genotype. A preceding modified leaf-gene expression might direct these Mn-dependent differences in the proteomic composition of the symplast. Therefore, the aim of this work was the investigation of Mn stress-induced transcriptomic differences in two cowpea genotypes differing in Mn tolerance. Additionally, Si, known to reduce the sensitivity of cowpea to excess Mn supply (Horst et al. 1999), was included to study early transcriptomic changes leading to Si-enhanced Mn tolerance in the Mn-sensitive cowpea genotype. Subtractive cDNA libraries were established by Suppression Subtractive Hybridization (Diatchenko et al. 1996).

Materials and methods

V. unguiculata L. Walp. cv. TVu 91 (Mn-sensitive) and cv. TVu 1987 (Mn-tolerant) were pre-cultured in the presence or absence of 20 µM Si for 14 days and afterwards treated with 50 µM Mn for one day, whereas control plants received 0.2 µM Mn continuously. Total RNA was extracted from the second oldest middle trifoliate leaf of every plant. The Super SMART™ PCR cDNA Synthesis Kit (Clontech, Palo Alto, CA) produced sufficient cDNA for the SSH approach (Diatchenko et al. 1996) using the PCR-Select™ cDNA Subtraction Kit (Clontech, Palo Alto, CA). Differentially expressed sequences were cloned into the pGEM®-T Easy Vector System (Promega, Madison, WI, USA). Selected recombinant clones were analysed by custom DNA sequencing (IIT Biotech GmbH, Bielefeld, Germany). Decontaminated sequences were searched against a nucleotidedatabase on the BLAST Server (Altschul et al. 1990).

Results

The PCR-based SSH technique enabled a selective enrichment and amplification of differentially expressed target cDNA fragments and the simultaneous suppression of non-target cDNAs. To investigate the beneficial effect of Si in the presence of Mn stress and its influence on the transcript level, a first bidirectional subtraction was performed (Figure 1 A, B). The cowpea cv. TVu 91 grown without Si under short-term excess Mn was compared to plants grown under Si and short-term enhanced Mn. Both libraries of the 1st SSH revealed a high influence of Si in combination with enhanced Mn supply on photosynthesis/respiration and metabolism. Under continuous Si supply in the presence of short-term Mn stress the downregulation of transcripts belonging to photosynthesis/respiration prevailed over their up-regulation. By contrast, the up-regulation of differentially expressed genes belonging to the metabolism fraction dominated over a downregulation. Differentially expressed genes coding for transport/cell motility processes were upregulated.

The constitutive effect of Si and its influence on the transcript level was studied in a second bidirectional subtraction (Figure 1 C, D) by comparing the cowpea cv. TVu 91 grown ± Si. Both libraries of the 2nd SSH revealed a high constitutive effect of Si on sequences related to photosynthesis/respiration. Under continuous Si supply the downregulation of transcripts belonging to photosynthesis/respiration prevailed over an upregulation. The amount of transcripts belonging to the functional category of metabolism was slightly reduced, and differentially expressed genes related to signal transduction, transport/cell motility and transcription/translation were upregulated by a continuous Si treatment of Mn-unstressed plants.

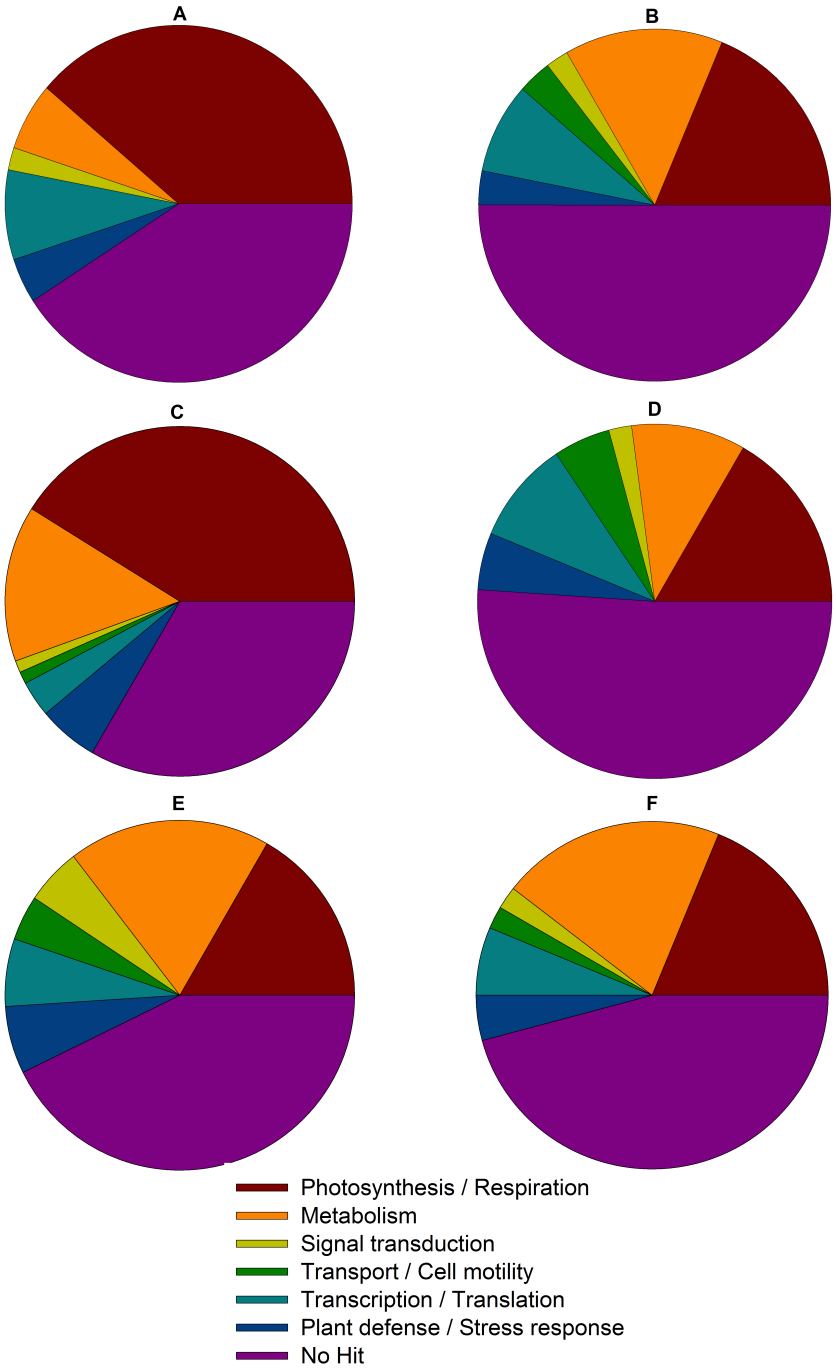

Figure 1: Changes in the specific transcriptome of the Mn-sensitive cowpea cv. TVu 91 and the Mn-tolerant cowpea cv. TVu 1987 as affected by optimum or short-term excess Mn treatment in combination with or without continuous Si supply. Gene expression products were categorized as described by the figure legend. Plants were pre-cultured in the presence or absence of 20 µM Si for 14 days and afterwards treated with 50 µM Mn for one day, whereas control plants received 0.2 µM Mn continuously. (A: 1st SSH: Downregulation of transcripts in TVu 91 by Si in combination with short-term enhanced Mn, B: 1st SSH: Upregulation of transcripts in TVu 91 by Si in combination with short-term enhanced Mn. C: 2nd SSH: Downregulation of transcripts in TVu 91 by Si, D: 2nd SSH: Upregulation of transcripts in TVu 91due to Si. E: 3rd SSH: Upregulation of transcripts in TVu 1987 by short-term enhanced Mn, F: 4th SSH: Upregulation of transcripts in TVu 91 by short-term enhanced Mn).

A third and fourth unidirectionally subtracted SSH were performed to analyse genotypic Mn-induced transcriptomic differences in both cowpea cultivars. Therefore, both cowpea genotypes were supplied with short-term excess Mn and compared to control plants (
Figure 1 E, F). The cDNA libraries indicated a major impact of Mn on the expression of genes belonging to photosynthesis/respiration, metabolism and transcription/translation The Mn-induced upregulation of genes belonging to these categories was slightly enhanced in the Mn-sensitive cowpea cv. TVu 91 compared with TVu 1987.

Discussion

A stress-related transcriptomic influence of Si was detected by comparison of the cowpea cv. TVu 91 grown under short-term excess Mn supply to Si-supplied plants, leading to considerable transcriptomic changes within the functional categories of photosynthesis/respiration and metabolism. The comparison of the continuously Si-supplied cowpea cv. TVu 91 to control plants indicated considerable Si-mediated transcriptomic changes in the functional categories of the photosynthesis/respiration, signal transduction, transport/cell motility and transcription/translation, reflecting a constitutive transcriptomic Si-effect. A consistent trend is reflected by the Si-mediated downregulation of photosynthesis/respiration-related sequences. Therefore, these findings indicate a high impact of Si particularly on the energy-providing pathways in both Mn-stressed and unstressed plants. A Si-based downregulation might affect potentially Mn excess-sensitive genes. A concomitant Si-based upregulation might in contrast affect tolerance-mediating genes. Finally, the Si-tuned interaction of up and downregulation of specific transcript categories could promote Mn tolerance in the Mn-sensitive cowpea genotype.
The comparison of both cowpea cvs. TVu 1987 and TVu 91 grown under optimum and elevated Mn supply clearly showed Mn-induced genotypic differences on the transcriptome level. Besides a major impact on photosynthesis/respiration and metabolism, a minor effect was also detectable in signal transduction, transport/cell motility, transcription/translation and plant defense/stress response, indicating a very early toxicity level in the Mn-sensitive cultivar. The same observations in the Mn-tolerant cultivar were rather unexpected since the ealier reported (Führs et al. 2008) strong induction of genes belonging into these functional categories after three days of excess Mn treatment already reflected the severity of an advanced Mn toxicity stage in cowpea cv. TVu 91. However, in general the results further emphasized the early stage of Mn toxicity development. Previously published results showing an enrichment of transcripts belonging to signal transduction and transport/cell motility especially in the Mn-sensitive cowpea genotype were supposed to be a first sign of Mn-induced stress response (Führs et al. 2008). In contrast, the strong induction of genes from this category might be a sign of a coordinated Mn-induced signal perception and signal transduction leading to apoplastic but also symplastic tolerance mechanisms only present in the Mn-tolerant cowpea cv. TVu 1987.
Current work includes an in-depth analysis of the constitutive and stress-combined transcriptomic influence of Si and a detailed analysis of Mn-induced genotypic changes on the transcript level based on the quantification of selected candidate-gene expression-patterns by qRT-PCR.

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