Impact of Silica on *Cucumber mosaic virus* infected cucumber *in vitro* plants

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BACKGROUND

- Silicon (Si) is omnipresent in soil, taken up via the roots as silicic acid, $Si(OH)_4$, and deposited in cell walls
- beneficial effects for crops and tissue culture: higher yield, mechanical strengthening, mitigation of pests, abiotic and biotic stress
- Si plays an active role in plant disease resistance in general
- fertilizers often contain Si to strengthen the plants
- previous studies focused on the role of Si with regard to different viral infections in tobacco implicating controversial effects for the host plant and accumulated Si content
- however, few molecular data is available on low Silicon accumulating plants and alleviation of viral diseases

→ **this study aims to provide information on changes in the gene expression of *in vitro* cultivated and Si supplemented *Cucumis sativus* plants to present candidate genes which are potentially involved in *Cucumber mosaic virus* (CMV) replication and defence.**

MATERIALS AND METHODS

- ***in vitro* culture regeneration** from *C. sativus* cultivar line B10 (**Fig 1**) was performed via leaf microexplants, cultivation w/o Si, NaCl
- 18 clones were generated in total
- 9 clones were mechanically **inoculated with CMV** (isolate PV-0187)
- **total RNA** was isolated 6 days post inoculation (dpi) (leaf/shoot), DNase treated and **mRNA enriched** by repeated polyT-oligonucleotide hybridization
- **RNA-Seq** was performed, CLC Genomics Workbench was used for mapping on the genomic draft of cucumber line B10
- **transcriptome analysis** was performed to obtain differentially expressed genes (**Table 1**)
- **quantitative (q) reverse transcription (RT)-polymerase chain reaction (PCR)** was performed: confirmation of RNA-Seq (control vs Si) (**Fig 3**) and analysis of CMV infected plants (CMV vs Si+CMV) (**Fig 4**).

RESULTS

- ✓ regeneration of cucumber line B10 was successfully performed
- ✓ infection of *in vitro* cultures with CMV was confirmed by RT-PCR
- ✓ RNA-Seq based on mRNA of control and Si supplemented re-generants provided data sets for transcriptome analysis
- ✓ 1.136 differentially expressed genes, beneficial for tissue cultures
- ✓ up- and down-regulated transcripts belong to primary and secon-dary metabolism
- ✓ qRT-PCR confirmed RNA-Seq results
- ✓ transcripts in cucumber altered through Si treatment implicate converse effects (promotional/suppressive) with regard to CMV infection

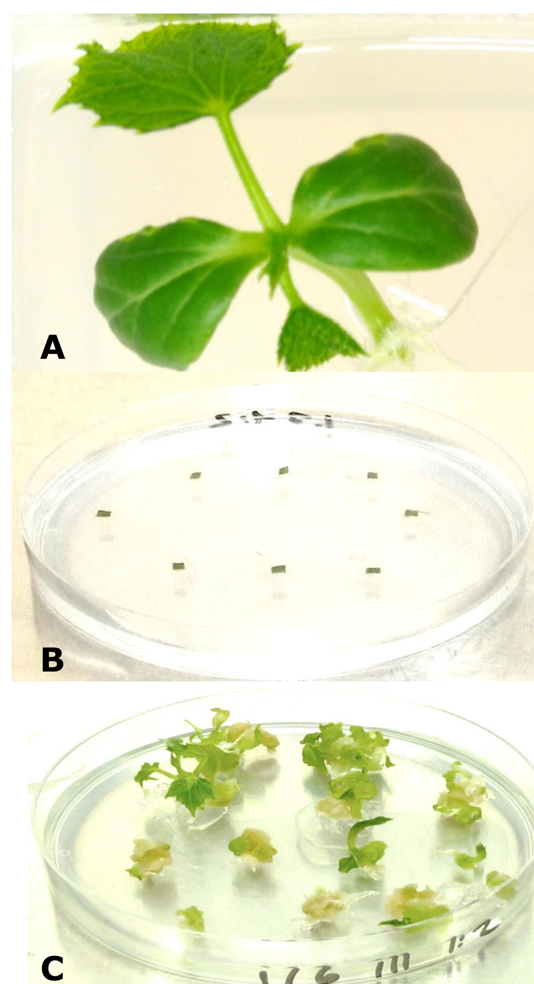
DIRECT REGENERATION OF *CUCUMIS SATIVUS* LINE B10

I. embryo sowing under aseptic conditions to obtain an *in vitro* plant cultivated on MS medium

II. first/second true leaf (**A**) were cut off for the preparation of leaf microexplants (**B**) for starting regeneration process to obtain genetically identical plants

III. calli division and propagation, under continuous treatment on MS medium:
- control (without supplements)
- sodium Silica ($Na_2O(SiO_2)_xxH_2O$), pH re-adjusted and medium containing NaCl
- NaCl (equal amounts as Si medium)

IV. calli with shoots and leaflets (**C**) prior transfer to rooting medium



V. rooting of regenerated plantlets

VI. transfer to new medium every two weeks, under continuous supplementation for complete rege-neration (eighteen homogenous clones in total) (**D**)

VII. mechanical CMV inoculation of leaves (nine clones)

VIII. 6dpi, RNA isolation of pooled aboveground (leaf/shoot) material (**E**) followed by DNase I treatment and mRNA enrichment; CMV infection was analyzed *via* RT-PCR (coat protein) on total RNA

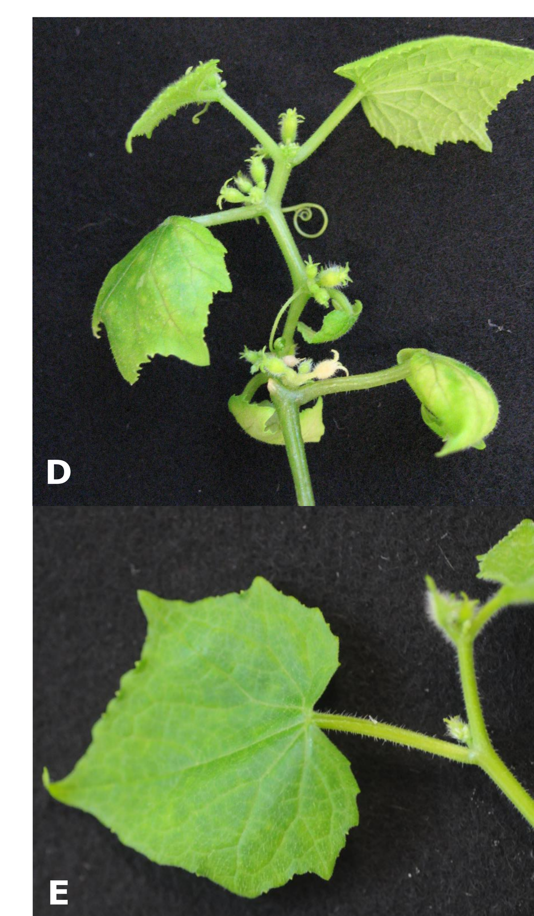


Figure 1. Different *in vitro* stages of *C. sativus* B10 direct regeneration process for isolation of total RNA and mRNA enrichment out of regenerated plants. The regenerants were cultivated with different supplements and partially infected with CMV. Pictures A to E shown here are excerpts from the regeneration experiment and represent the key steps.

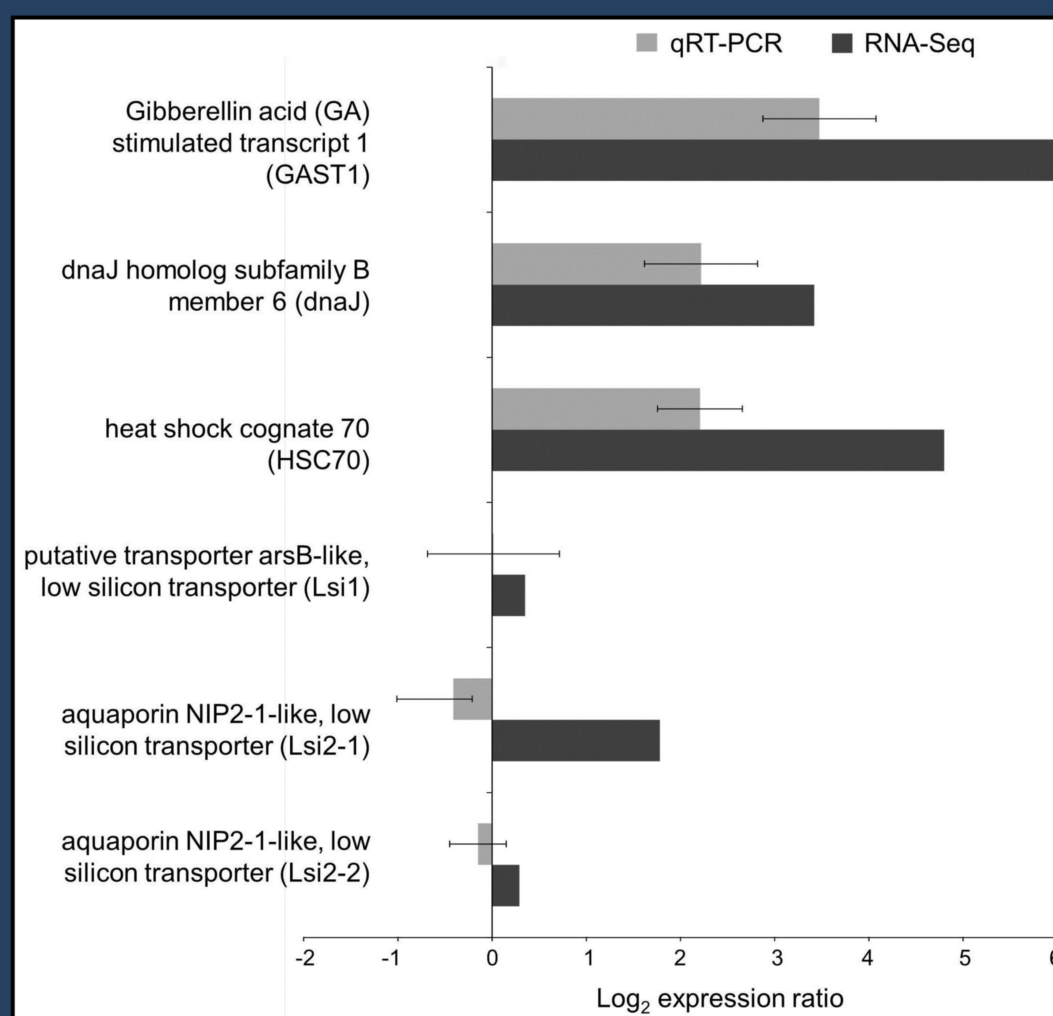
TRANSCRIPTOME ANALYSIS

- RNA-Seq: performed on mRNA derived from three control and three Si-treated samples (pooled leaf & shoot material)
- each 19,000 transcripts from 19,896 genes determined
- on average: each predicted gene is assigned to one transcript
- 1,136 differentially expressed genes (DEGs) at $P < 0.01$ and ≥ 1.5-fold change
- 572 genes up-regulated, 564 genes down-regulated
- higher calculated amount of DEGs than expressed *in planta*
- transcripts belong predominantly to primary metabolism

→ ***supports previous studies on benefits of (sodium) silica for tissue culture***

Table 1. Selected differentially expressed genes through sodium Si treatment in cucumber and their roles in plant metabolism obtained from the calculation. * indicates possible strenghtened effect through Si supplementation towards NaCl traces (abiotic stress).

gene	description	accession number	fold change	role
GAST1	Gibberellin acid (GA) stimulated transcript 1	LOC101223935	75	GA metabolism, up-regulated by GA or NaCl*
ATM1-2	ammonium transporter 1 member 2	LOC101227720	27	ammonium uptake from soil
dnaJ	dnaJ homolog subfamily B member 6	LOC101224788	25	up-regulated by NaCl*, viral replication, plant development
MLP 328	MLP-like protein 328	LOC101232410	17	defense response
LOX1.5	linoleate 9S-lipoxygenase 5	LOC101230344	17	pathogen resistance
CSLG3	cellulose synthase G3	LOC101212740	8	polymerization of hemicellulose
EXPB1	Expansin-B1	LOC101204155	-35	senescence delay



VALIDATION OF TRANSCRIPTOME BY QRT-PCR

Figure 3. Validation of gene expression regulated by Si obtained by RNA-Seq performing qRT-PCR on selected genes. The results show the comparison between the log_2 fold change in the gene expression obtained by RNA-Seq and qRT-PCR. The data presented is the average of two (control) and three (sodium Si treated) independent biological replicates, and technically repeated twice. *Adenosine phospho-ribosyltransferase* was used as endogenous control, and an intronic sequence for confirmation of successful DNA removal. Error bars represent the standard deviation (qRT-PCR).

→ ***consistent pattern for four genes***

→ ***higher tendency for values from transcriptome analysis***

→ ***RNA-Seq confirmed by qRT-PCR***

QRT-PCR ON SELECTED GENES RELATED TO CMV

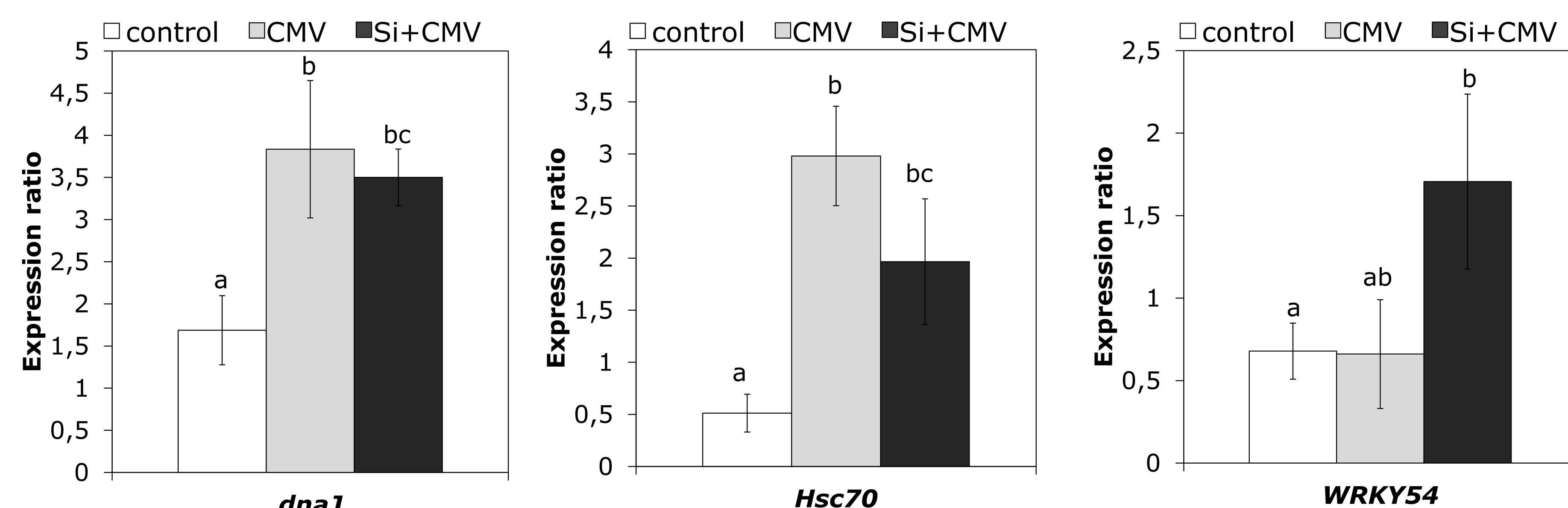


Figure 4. Expression analysis of selected cucumber genes involved in CMV replication (*dnaJ*, *Hsc70*) and defence (*WRKY54*) by qRT-PCR. 1-step qRT-PCR was performed using *APRT* as endogenous control for all treatments. The data presented is the average of three independent biological replicates and technically repeated three times. Error bars represent the standard deviation. The statistical analysis was performed by ANOVA followed by Tukey's honest significance difference test ($p < 0,05$). Different letters above the bars indicate significant differences ($p < 0.05$).

→ ***Si supplementation leads to alteration of gene expression related to CMV***
→ ***Si may not induce tolerance towards the CMV infection based on transcript level***

SUMMARY

- ✓ cucumber transcriptome under sodium silica supplementation highlights beneficial role of Si for tissue cultures examined on aboveground material
- ✓ qRT-PCR confirmed RNA-Seq with higher values from transcriptome calculation
- ✓ Si influences gene expression involved in CMV replication (*dnaJ*, *Hsc70*) and defence (*WRKY54*) with limited impact six days post inoculation in cucumber cultures
- ✓ neutral effect due to Si treatment likely which was investigated on selected cucumber transcripts related to CMV infection
- ✓ Si may not be suitable for tolerance induction towards viruses in *in vitro* cucumber

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