Impact of silica supplementation on virus infected cucumber cultures

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Background

- Silicon is omnipresent in soil, taken up via the roots as silicic acid, $Si(OH)_4$ (Si), and deposited in cell walls
- beneficial effects for plants: higher yield, mechanical strengthening, mitigation of pests, abiotic and biotic stresses
- Silicon plays an important and active role in plant disease resistance in general
- fertilizers often contain Silicon 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 gene expression of *in vitro* cultivated and Si supplemented *Cucumis sativus* plants to present candidate genes which are putatively involved in *Cucumber mosaic virus* (CMV) 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
- **quantitative (q) reverse transcription (RT)-polymerase chain reaction (PCR)** was performed: confirmation of RNA-Seq (control vs Si) and analysis of CMV infected plants (CMV vs Si+CMV) (**Fig 3**).

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 regenerants provided data sets for transcriptome analysis
- ✓ 1.136 differentially expressed genes ($P < 0.01$, ≥ 1.5 fold change)
- ✓ up- and down-regulated transcripts belong to primary and secondary metabolism
- ✓ qRT-PCR confirmed RNA-Seq results
- ✓ transcripts in the cucumber transcriptome due to Si treatment implicate converse effects (promotional/suppressive) with regard to CMV infection

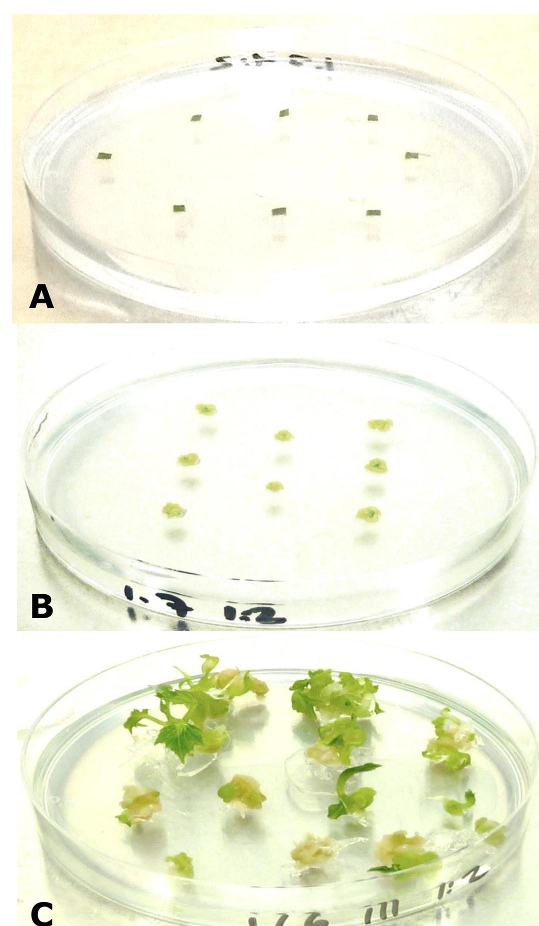
From *C. sativus* line B10 direct regeneration of plants to mRNA:

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

II. preparing of leaf microexplants from first/second true leaf (**A**) for starting regeneration process to obtain genetically identical plants

III. calli division and propagation (**B**), under continuous treatment on MS medium:
- control (without supplements)
- Silica ($Na_2O(SiO_2)_x xH_2O$), pH re-adjusted and medium containing NaCl
- NaCl

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



V. rooting of regenerated plants (**D**), 18 homogenous clones in total

VI. mechanical CMV inoculation of leaves (9 clones)

VII. 6dpi, RNA isolation of pooled aboveground (**leaf/shoot**) material followed by DNase treatment; CMV infection was analyzed *via* RT-PCR

VIII. mRNA enrichment for RNA-Seq/qPCR (**E**), quality check *via* gelelectrophoresis and quantitation *via* NanoDrop measurement

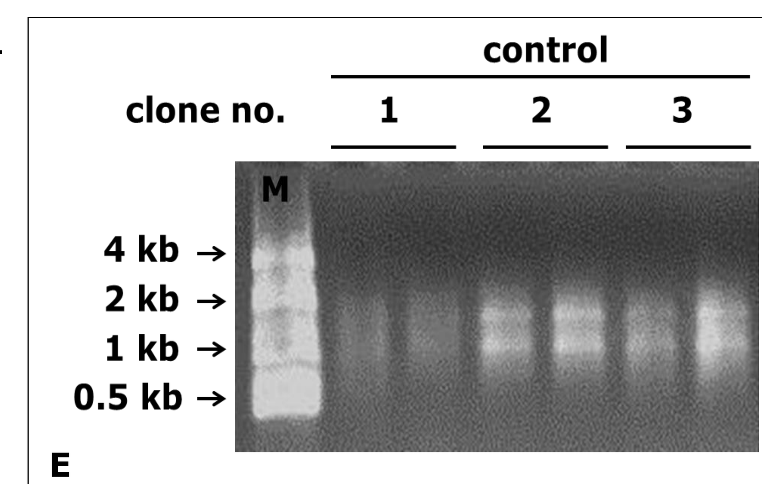


Figure 1. Different *in vitro* stages of *C. sativus* B10 direct regeneration process for isolation of total RNA and mRNA enrichment out of regenerated plants. The regenerants were cultivated with different supplements and partially infected with CMV. Pictures A to E shown here are excerpts from the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

Transcriptome approach

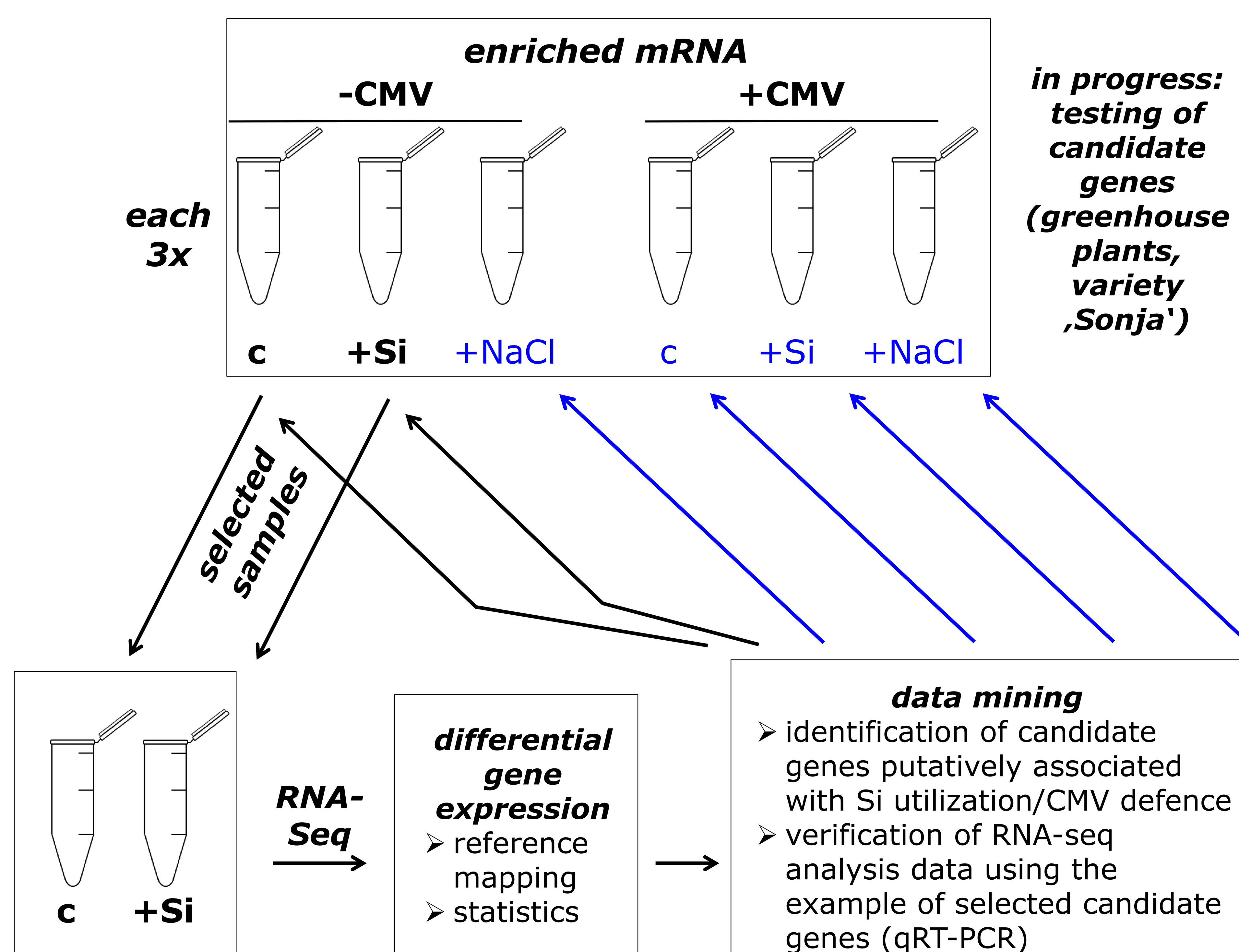


Figure 2. Experimental set-up for transcriptome approach. *C. sativus* clones were cultivated *in vitro*. Per treatment, 3 clones were obtained (18 clones in total). Control plants (c) were cultivated on non-modified Murashige and Skoog medium, whereas 2 mM silicic acid (+Si) and NaCl respectively were added to medium. Half of the clones were mechanically inoculated with CMV. 6 dpi, leaf and shoot samples from each clones were taken for total RNA isolation followed by mRNA enrichment. mRNA samples were taken from the non infected control- and Si-group (6 samples in total) for RNA-Seq performed with the Illumina platform (black). Reference mapping to B10 draft genome and differential gene expression was calculated using CLC Genomics workbench. Genes associated with Si utilization and CMV defence are analyzed to confirm RNA-Seq results (black) and in the other 4 treatment groups using mRNA for qRT-PCR (blue). Testing of candidate genes from greenhouse cultivated plants is in progress.

RNA-Seq results

- 19,000 transcripts from 19,896 genes determined
  - on average: each predicted gene is assigned to one transcript
- 1,136 differentially expressed genes at $P < 0.01$ and ≥ 1.5-fold change; slightly up-/down-regulation due to NaCl traces in medium possible
  - 572 genes up-regulated, 564 genes down-regulated
  - transcripts belong to primary and secondary metabolism
  - host candidate genes with reference to CMV infection:
    - *dnaJ* (support of replication due to interaction with replication complex)
  - *WRKY14* (homolog to *GhWRKY15*; promotes resistance against viruses)
  - *WRKY54* (homolog to *AtWRKY70*; interacts with protein, resistance to CMV yellow strain 1 [RCY1])

qRT-PCR

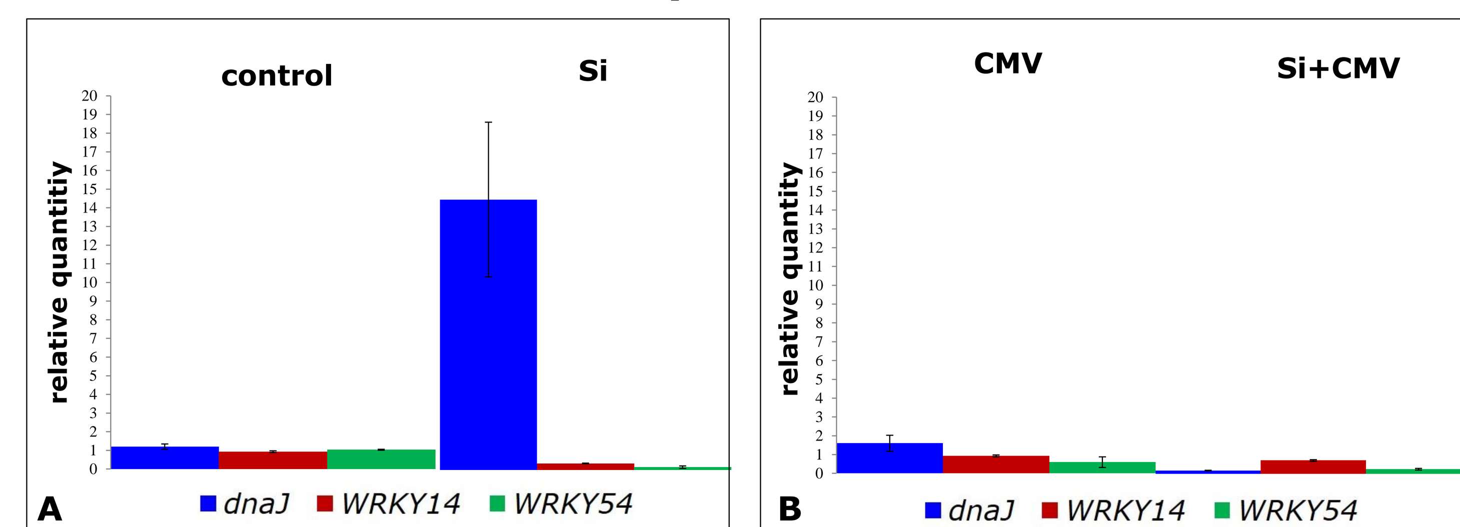


Figure 3. qRT-PCR on selected cucumber genes involved in CMV defence responses: **A** control vs Si set for confirmation of RNA-Seq, **B** analysis of Si supplemented and CMV infected plant material. 1-step qRT-PCR was performed using adenine phosphoribosyltransferase (APRT) as endogenous control for all treatments. Duplicates of two mRNA samples per gene were analyzed. Data are presented as the mean±standard deviation.

Summary

✓ RNA-Seq results were confirmed by qRT-PCR on selected genes

➢ up-regulation of *dnaJ* may support replication of CMV if infected

➢ down-regulation of *WRKY14/ WRKY54* due to Si treatment may suppress CMV resistance if infected

➢ down-regulation of *dnaJ* due to Si treatment and following CMV infection may suppress CMV replication and promotes resistance towards CMV

→ ***converse effects due to Si treatment based on the cucumber transcriptome with regard to CMV infection***

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