Impact of Silica Supplementation on Cucumber Transcriptome

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Background

- Silicon (Si) is omnipresent in the soil, taken up via the roots as silicic acid, $Si(OH)_4$ (Si), and finally deposited in cell walls
- beneficial effects for plants: higher yield, mechanical strengthening, mitigation of pests, abiotic and biotic stresses
- Si plays an important and 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 stresses such as salt, pathogens or pests; mainly Si accumulators and monocots such as rice and wheat
- however, few molecular data is available on low Si accumulating and non-stressed plants at a late developmental stage

→ this study aims to provide information on changes in gene expression of *in vitro* cultivated, Si supplemented and non-stressed *Cucumis sativus* plants to analyse genes involved in Si utilisation and disease resistance

Materials and Methods

- ***in vitro* culture regeneration** from *C. sativus* cultivar line B10 (**Fig 1**) was performed *via* leaf microexplants; clones were cultivated on non-treated (control) or sodium Silica-treated Murashige Skoog (MS) medium, and rooted
- three clones per treatment were selected (control, Silica)
- **total RNA** was isolated (leaf/shoot), Dnase I treated and **mRNA enriched** by repeated polyT-oligonucleotide hybridization
- **RNA-Seq** (Illumina) was performed, CLC Genomics Workbench was used for mapping on the genomic draft of cucumber line B10 as well as for **transcriptome analysis** and **empirical analysis of differentially expressed genes (DEGs)**
- **functional analyses of DEGs** was performed by comparing deduced amino acid sequences against InterPro database
- **quantitative (q) reverse transcription (RT)-polymerase chain reaction (PCR)** was performed on selected genes for confirmation of RNA-Seq

Results

✓regeneration of cucumber line B10 was successfully performed

✓RNA-Seq based on mRNA of control and Si supplemented re-generants 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, some assigned to traces of NaCl in medium

✓qRT-PCR confirmed RNA-Seq results

✓transcripts of Si treated cucumber support previous reported beneficial effects through Si supplementation

✓basis for Silicon induced disease resistance in a dicot

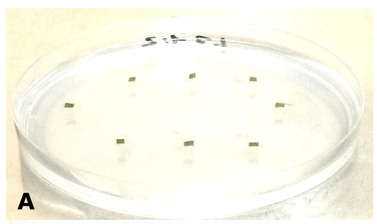
***Cucumis sativus* line B10 direct regeneration of plants for obtaining mRNA**

(1) embryo sowing under aseptic conditions to obtain an *in vitro* plant cultivated on Murashige and Skoog medium

(2) preparing of leaf microexplants from first/second true leaf (**A**) for starting regeneration process to obtain genetically identical plants in the dark

(3) calli division and propagation under light (**B**), continuous treatment on MS medium:
- control (without supplements)
- Silica ($Na_2O(SiO_2)_xxH_2O$), pH re-adjusted and medium containing traces of NaCl

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



(5) rooting of regenerated plants using indole acetic acid (**D**) (three homogenous clones per treatment, six in total)

(6) total RNA isolation of pooled aboveground (**leaf/shoot**) material (two samples per clone) followed by DNase I treatment

(7) mRNA enrichment for RNA-Seq, quality check *via* gelelectrophoresis (**E**) and quantitation *via* NanoDrop measurement

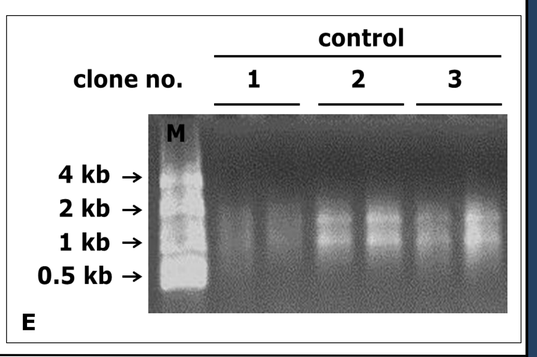


Figure 1. Scheme presenting different *in vitro* stages of *Cucumis sativus* B10 direct regeneration process for isolation of total RNA and mRNA enrichment. The regenerants were cultivated on medium with different supplements and rooted. Pictures A to D shown here are excerpts from the regeneration experiment and represent the key steps, picure E shows a gel image of mRNA enrichment.

RNA-Seq and Transcriptome Profiling

Control

- 1.2, 1.5 and 1.6 million 94b-reads obtained from three clones (43,341,194 reads in total)
- duplicate reads removed, 14,952,897 reads remaining
- 18,957 transcripts from 19,896 reference genes determined (95.3%)

Silica

- 1.4, 1.4 and 1.5 million 94b-reads obtained from three clones (44,315,629 reads in total)
- duplicate reads removed, 15,439,386 reads remaining
- 18,882 transcripts from 19,896 reference genes determined (94.9%)

Analysis of Differential Gene Expression:

- 1,136 differentially expressed genes at $P < 0.01$ and ≥ 1.5-fold change
  - 572 genes upregulated, 564 genes downregulated
  - identified transcripts refer to primary and secondary metabolism
  - genes regulated by NaCl traces in Si medium may explain high number of DEGs

Functional Analysis of Differentially Expressed Genes

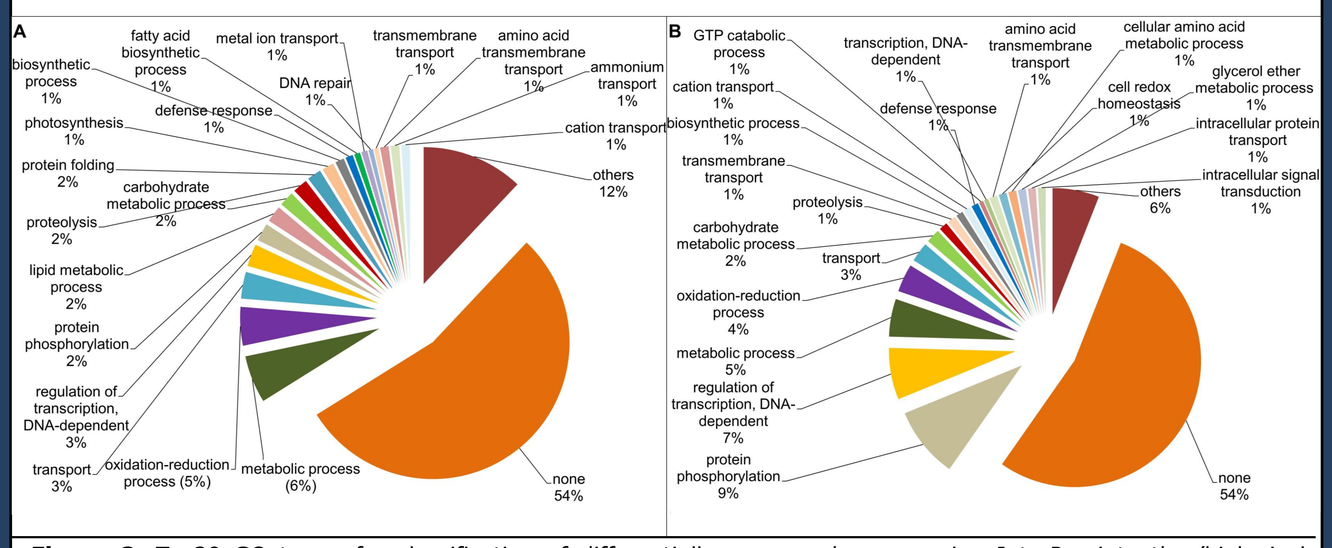
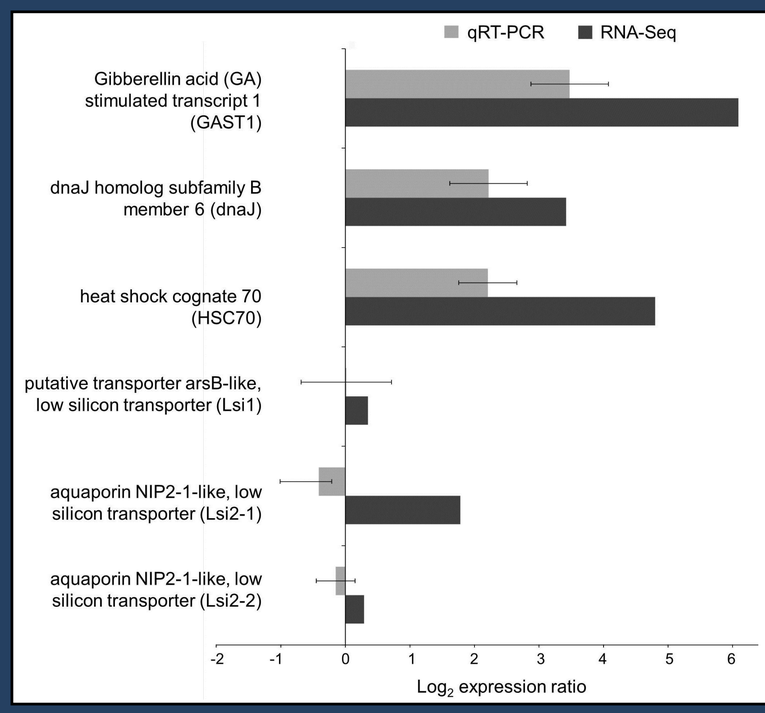


Figure 2. Top20 GO terms for classification of differentially expressed genes using InterPro into the 'biological process' category. Represented are the Top20 GO results from (A) upregulated transcripts and (B) downregulated transcripts, in the silica group compared to the control. Other terms, in addition to the Top20, are summarised.

Table 1. Selected differentially expressed genes through Si treatment and their roles in plant metabolism. * 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
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 RNA-Seq results 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 are means of two (control) and three (sodium Si treated) biological replicates, and technically repeated twice. *Adenosine phosphoribosyltransferase* was used as endogenous control, and an intronic sequence for confirmation of successful DNA removal. Error bars represent the standard error (qRT-PCR).

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

→ ***RNA-Seq confirmed by qRT-PCR***

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

Summary

- ✓ *in vitro*-generated clonal cucumber plants were successfully generated
- ✓ 18,957 (control) and 18,882 (Silica) cucumber transcripts referring to 19,896 genes were identified
- ✓ 1,136 differentially expressed genes determined; some assigned to traces of NaCl
- ✓ transcripts belong to biological processes: defence against abiotic and biotic stresses, cell wall modification
- ✓ RNA-Seq results were confirmed by qRT-PCR on selected genes
- ✓ transcriptome data of non-stressed, Si treated cucumber support previous reports on positive effects through Si

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Acknowledgements

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