**Impact of Silicon 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, and finally deposited in cell walls.
- Silicon benefits 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 are available on low Si accumulating and non-stressed plants at a late developmental stage.

**Materials and Methods**

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

**RESULTS**

- Regulation of cucumber line B10 was successfully performed.
- RNA-Seq based on mRNA of control and Si supplemented regenerated plants 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 microplants 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)
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 leaf/shoot (E) (material samples per clone) followed by DNase I treatment
7. mRNA enrichment for RNA-Seq, quality check via gelelectrophoresis (F) and quantization via NanoDrop measurement

**Figure 1. Scheme presenting different in vitro stages of Cucumis sativus B10 direct regeneration process for isolation of total RNA and RNA 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, picture E shows a gel image of RNA enrichment.**

**Table 1. Selected differentially expressed genes through Si treatment and their roles in plant metabolism.** ^a^n indicates possible strengthened effect through Si supplementation towards NaCl traces (abiotic stress).**

<table>
<thead>
<tr>
<th>gene</th>
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<td>GAST1</td>
<td>Gibberellic acid (GA) stimulated transporter</td>
<td>LOC101223935</td>
<td>75</td>
<td>GA metabolism, up-regulated by GA or NaCl</td>
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<td>ATM1-2</td>
<td>ammmonium transporter 1</td>
<td>LOC101222770</td>
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<td>ammmonium uptake from soil</td>
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<td>dHA1</td>
<td>dha homoligod subtility B</td>
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<td>up-regulated by NaCl, viral replication</td>
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<td>Mep-like protein 32</td>
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<td>LOC101204155</td>
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</table>

**Functional Analysis of Differentially Expressed Genes**

**Figure 2. Top20 GO terms for classification of differentially expressed genes using InterPro into the biological processes category. Represented are the Top20 GO results from (A) up-regulated transcripts and (B) down-regulated transcripts, in the silica group compared to the control. Other terms, in addition to the Top20, are summarised.**

**References**


**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 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 intrinsic 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 *in vitro*-generated clonal cucumber plants were successfully generated
- 18,997 (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
- transcriptions 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.