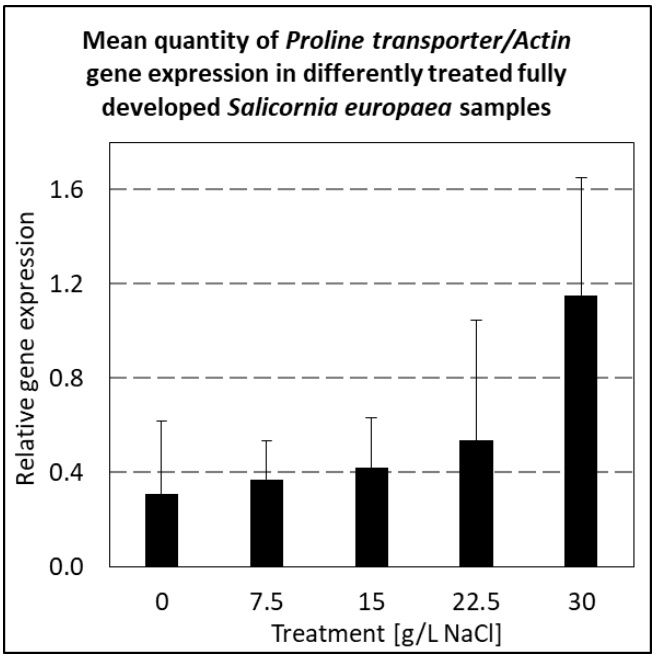

Gene expression analysis in salt-tolerant plant species – Example *Salicornia europaea*

After optimization of RNA extraction, cDNA synthesis and qPCR conditions from plant material containing high concentrations of salt gene expression analysis can be performed in a reliable way.

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The circular approach of AQUACOMBINE combines aquaculture, farming and bioprocessing to utilize all fractions of the produced biomass and produce value added food, feed, bio-compounds and bioenergy.

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After optimization of RNA extraction, cDNA synthesis and qPCR conditions from plant material containing high concentrations of salt gene expression analysis can be done in a reliable way.

Background: An increase of salinity in the hydroponic culture might induce stress; stress might induce secondary compounds and influence the overall chemical composition of the plants; stress might also decrease the biomass and therefore the yield. Technically easy methods to analyze the stress grade at an early stage are important to adjust and modify cultivation conditions to obtain large amounts of biomass containing sufficient amounts of products for biorefinery. Gene expression analysis was established as a sensitive method to detect stress levels at an early stage.
Methods: Deep-frozen plant material was mortared in liquid nitrogen to a fine powder. RNA isolation and reverse transcription were performed using a commercial kit for RNA isolation from plants. To remove DNA a DNase treatment was performed twice. Synthesis of cDNA was performed with approximately 250 ng of total RNA, 50 pmol of random nonamer primer, 10 pmol oligo-dT primer, 200 units of Moloney murine leukemia virus reverse transcriptase and 1 mM deoxyribonucleotide triphosphates in reaction buffer. Specific primers based on literature and sequence analysis were designed for qPCR. Several putative reference genes were tested.
Results: *Actin* and *UBC* performed most equally at different salinities, *UBC* and *UBQ* were most suited at different developmental stages. Expression levels of genes involved in adaptation to various environmental stresses - especially salt-related stress - and involved in different metabolic pathways relevant for valuable products were investigated. One of these gene codes for a proline transporter which is responsible for the distribution of the related osmoprotectant. Many plant species synthesize L-proline in the cytosol and accumulate it in chloroplasts. The accumulation of L-proline in plants is a well-known physiological response to osmotic stress caused by salinity, drought, and other abiotic stresses. We found with an increase in salinity increasing expression levels of the *proline transporter* gene in *Salicornia europaea* that seems to be a perfect marker gene for the influence of salinity.

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