Transcriptomic and Proteomic Analysis of Ecotoxic Modes of Action in *Myriophyllum spicatum*

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Background

- The molecular effects and the underlying mode of action (MoA) of substances are of increasing interest in the ecotoxicological risk assessment
- From such data, biomarkers for specific MoA may be derived and used in future screenings to aid not only in the assessment, but also early developmental stages of new substances
- The application of OMICs techniques on organisms used in ecotoxicological studies enables insights into these molecular effects and yields unique molecular fingerprints for substances
- In this study, *Myriophyllum spicatum*, among other species used in the evaluation of pesticide ecotoxicity on primary aquatic producers, was exposed to the pharmaceutical atorvastatin (AV) and pesticide bentazon (BT)
  - AV is a known 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) inhibitor, BT a photosystem II (PSII) inhibitor
- Based on a shortened approach of the OECD test guideline 239[1], the molecular impact of low effect concentrations on *M. spicatum* was studied via transcriptomics and proteomics

Methods and data analysis

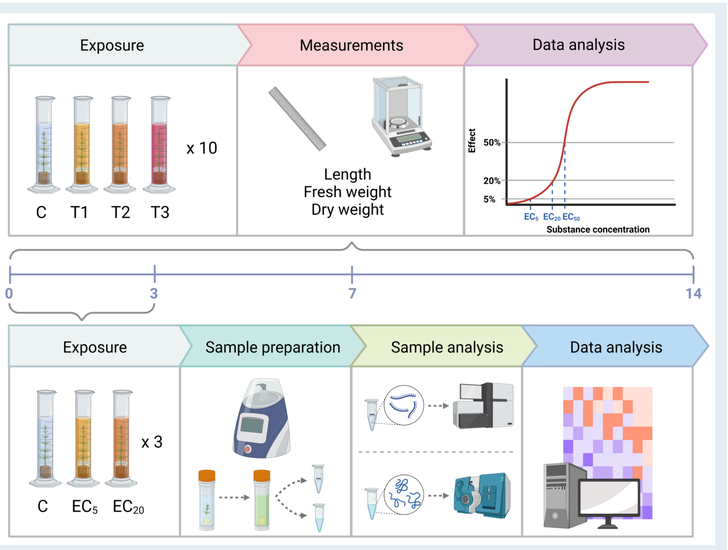

Figure 1: Workflow of the pre-test to determine low-effect concentrations and the shortened 3-day assay with Myriophyllum spicatum. EC_5 and EC_{20} were chosen based on the growth rate of the total shoot length. Created with biorender.com

- *M. spicatum plants* were exposed for 3 days to low-effect concentrations of AV and BT in a shortened assay based on the OECD test guideline 239 (see figure 1)
- Both RNA and protein were extracted from the fresh plant heads in a coupled extraction and analyzed via RNA-seq and MS/MS analysis
- As there is no available reference genome for *M. spicatum*, a *de novo* assembly of the RNA-seq data using Trinity was performed and the results were annotated via Trinotate
- Differentially expressed genes (DEGs) were determined with DESeq2 after RSEM quantification
- Overrepresentation analysis of pathways was performed via Goseq
- Proteomic data were mapped against the translated Trinity assembly and differentially abundant proteins are currently being analyzed

Results

- 52,000 and 54,000 transcripts could be assembled for atorvastatin and Bentazon, respectively, with a shared universe of 46,000 genes
  - 53% of the genes for AV and 54% for BT could be annotated using the Trinotate pipeline

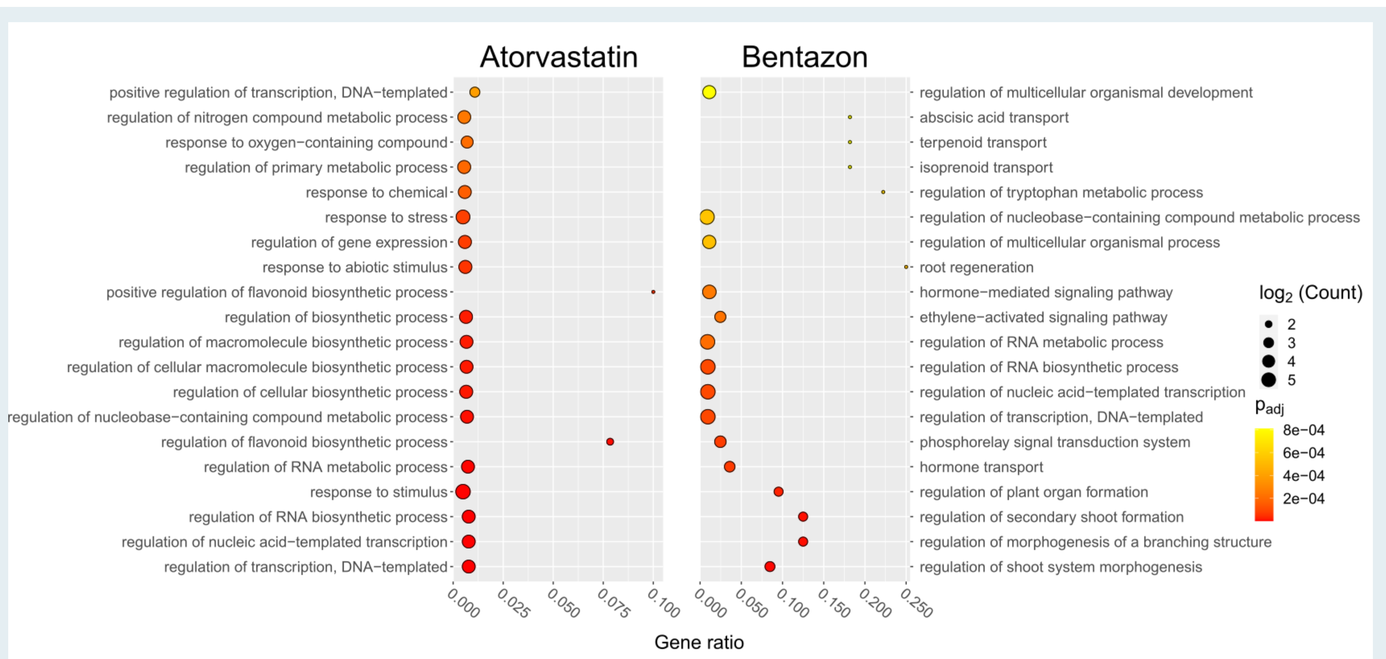

Figure 2: Overrepresentation analysis with Goseq of differentially expressed genes following the exposure of M. spicatum with Atorvastatin and Bentazon. Shown are the enriched biological processes in the overlap of a 3-day exposure with the respective EC_5 and EC_{20} concentrations.

Results

- A strong correlation between the substance concentration and the DEG count as well as the DEG overlap could be observed for both AV and BT
  - Almost no overlap and correlation between AV and BT DEGs, only 11 shared DEGs between all exposures
  - The common subset of EC_5 and EC_{20} exposures can therefore be seen as a substance- and MoA-specific molecular response and not just generic stress responses
- In the overrepresentation analysis, many of the 164 enriched processes found in both exposures to AV were related to the regulation of biosynthetic processes, among those steroid and isoprenoid pathways
  - As AV targets HMGR, a key enzyme of the mevalonate pathway, this disruption of biosynthetic processes matches the expectations for the molecular effects of the substance
  - In line with a previous study in *Lemna minor*[2], processes related to abscisic acid, reported to mediate regulation of HMGR, were affected in the higher concentration
  - Similarly, the cellular response to lipids was highly enriched in both plants. Unlike in *L. minor* however, no processes related to ethylene were highly enriched
- For the PSII inhibitor BT, among the 192 affected processes in the DEG overlap were, as anticipated, cellular responses to blue and far red light
  - Furthermore, matching results from *L. minor*, processes related to oxidative stress were found to be enriched by the higher exposure of BT, hinting at the inhibition of the PSII and therefore the release of toxic radicals
  - The observed enrichment of processes related to plant organ and shoot formation as well as morphogenesis may be early stress responses by the plant due to the lack of photosynthetic products

Conclusion

- The application of OMICs techniques to *M. spicatum* revealed distinct, concentration-dependent molecular responses to two substances with a differing MoA after only 3 days of exposure
- Pathways enriched by exposure match with the reported molecular effects of bentazon and atorvastatin
- Biomarkers specific for the MoA of each substance may be derived from this dataset for future studies for early predictions of toxic effects on organisms

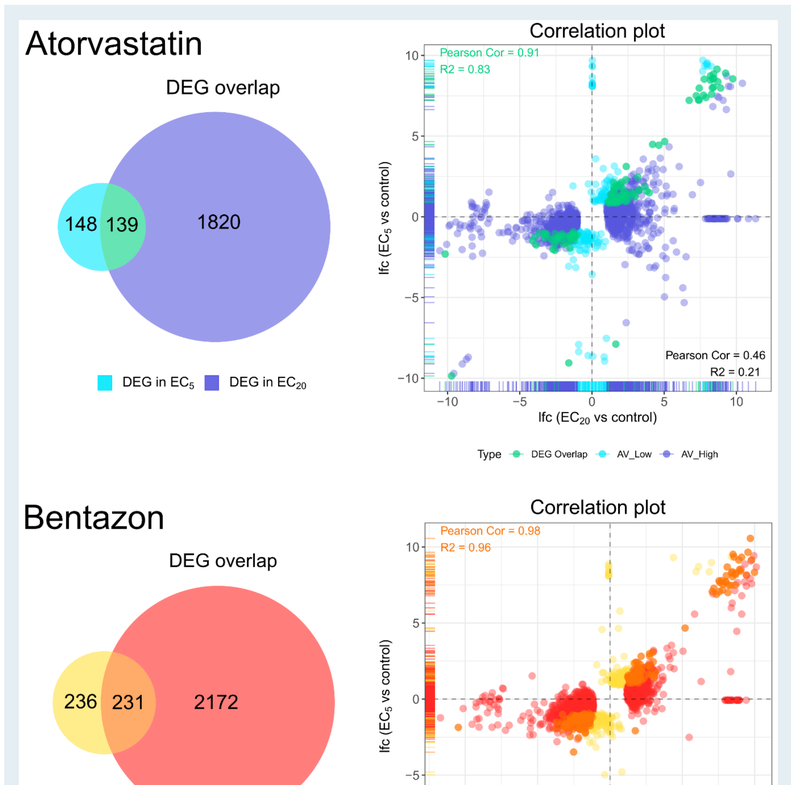

Figure 3: Differentially expressed genes (DEGs) in M. spicatum following the 3-day exposure with EC_5 and EC_{20} concentrations. Shown is the overlap and correlation of both exposure conditions as well as both substances. lfc = log fold change.

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1 OECD (2014): Test No. 239: Water-Sediment Myriophyllum Spicatum Toxicity Test. Available online at https://www.oecd-ilibrary.org/content/publication/9789264224155-en.

2 Loll, Alexandra; Reinwald, Hannes; Ayobahan, Steve U.; Göckener, Bernd; Salinas, Gabriela; Schäfers, Christoph et al. (2022): Short-Term Test for Toxicogenomic Analysis of Ecotoxic Modes of Action in Lemna minor. In *Environmental science & technology* 56 (16), pp. 11504–11515. DOI: 10.1021/acs.est.2c01777.