"roundup" and module differential analysis (moda)

Environment, Plants

The broad-spectrum glyphosate-based herbicide Roundup had witnessed a tremendous increase in use since its introduction in the mid 1970's as a means of weed growth control, especially during attempts to counteract the emergence of resistant weeds which was then followed by the introduction of genetically engineered resistant crops. The scope of its ecological impact had thus been a constant subject of concern for the scientific community with more recent studies reconsidering previous conclusions regarding the actual half life of Roundup's active ingredients and metabolites, its presence and persistence in the environment as well as the existence of targeted pathways outside of plants, algae, fungi and some bacteria as initially speculated.

Moreover, along with the direct contact of some approved glyphosate-based pesticides with aquatic environments, the sheer amount of Roundup usage poses a definite risk of water contamination. In a study exposing mussels to environmentally relevant concentrations of glyphosate, enrichment analysis pointed to the toxicant's adverse effects on key biological pathways including endoplasmic reticulum stress response, cell signaling, metabolism and Ca^{2+} homeostasis. Oxidative stress was also frequently reported across studies including a few conducted by de Menezes et al. on a number of fish species which found an increase in thiobarbituric acid reactive species, an indicator of oxidative stress. Additionally, significantly higher levels of superoxide dismutase antioxidant enzymes in the freshwater blackworm species as well as decreasing amounts of Glutathione S-transferase, a detoxification metabolic enzyme, in a species of south American tadpoles, both indicated oxidative stress responses.

Reproduction of small crustacean species were also reported to have been affected. A significant decrease in reproductive rates was reported for Simocephalus vetulus, a common wetland crustacean zooplankton, exposed to the glyphosate-based herbicide Visionn as well as for M. micrura, another small crustacean zooplankton, exposed to glyphosate. These results are comparable to those of vertebrates and more complex invertebrates where, for example, levels of steroidogenic factor 1 in fish, which is a transcription factor for several reproduction-related genes, increased with glyphosate exposure and coincided with an abnormal growth of oocytes. Furthermore, chronic exposure of an estuarine crab to both glyphosate and Roundup had led to the impediment of ovarian growth, where glyphosate significantly inhibited ovarian protein synthesis. One study also monitored the hatching dynamics of 30 taxa of zooplankton egg banks in the presence of another glyphosate-based herbicide and noted the decrease in species diversity at a certain concentration of the toxicant where cladocerans, the order of crustacean water flees, decreased in relative proportion.

Although many studies were published regarding the toxicity of glyphosate-based herbicides on aquatic species including those relevant to freshwater ecosystems, there is a lack of understanding regarding the molecular mechanisms by which these common pesticides exert their toxicological effects. Therefore, the utilization of a suitable model organism for freshwater ecosystems such as the Daphnia species in conjunction with advanced omics and multiomics tools would be extremely valuable in investigations that aim to understand the molecular targets of ecological toxicants and their mode of action.

Daphnia, commonly known as water fleas, are a crustacean genus of over 200 mirco- and macroscopic species of zooplanktons that inhabit a wide range of freshwater bodies around the world. They are mostly pelagic and typically filter feeders that graze on phytoplankton including algae and bacteria. There are a number of traits that recognized Daphnia as an especially important model organism for the currently advancing fields of ecology, evolution, toxicology and their interrelated disciplines. Firstly, they are keystone species in pelagic freshwater ecosystems, meaning that they partake in a crucial role driving the community dynamics more so than the other species in their habitat and have a highly significant influence in the aquatic food-webs. They are also hosts to a wide variety of parasites. Playing the role of a strong community interactor and a genus at the center of aquatic food webs make them valuable ecogenomic models.

Daphnia commonly engage in cyclic parthenogenesis as a mode of life cycle and reproduction, where females asexually produce diploid eggs during growth which will directly develop into adults. This persists until the population grows to include genetically identical individuals. Under unfavorable conditions however, females produce haploid eggs which, after fertilization, are encapsulated by an ephippium that can protect against sever conditions such as drought and temperature. Interestingly, this creates dormant banks where the eggs are preserved in sediments over many decades as zooplanktonic fossils. As a result, the life cycle of Daphnia presents two main advantages. First, their clonal reproduction aids in eliminating confounding factors of varying genetic backgrounds, a characteristic that is hard to attain with other model organisms and secondly, the dormant egg banks can serve to follow evolutionary processes in resurrection studies to get a better insight into eco-evolutionary dynamics. Additionally, Daphnia has a wealth of information regarding its phenotypic and genotypic variability, its physiology, population dynamics and ecological and evolutionary responses.

The relationship between Daphnia and their microbiome is yet another aspect of interest. It was clearly shown by Sison-Mangus, M. P., et al. and further confirmed by Mushegian, A. and Ebert, D. that the absence of the microbiome resulted in significant adverse effects on fitness including slower growth, smaller size, lower reproducibility and higher mortality, all of which were preventable with the restoration of the microbiome. Additionally, Mushegian, A., et al. demonstrated that the microbiome facilitates healthy hatching and embryonic development of the animal, whereas Macke, E., et al. pointed to the adaptive importance of the gut microbiome, in the context of tolerance to cyanobacteria toxicity. This, and it has long been shown in Drosophila species, whose gut flora are comparable to that of Daphnia, that genes underlying a certain group of functions including immunity, metabolism and development are partially regulated by the gut microbiome, where another study analyzing the transcriptional networks showed that the co-expression of those genes is also enhanced by the bacteria.

While it is important to experiment on appropriate model organisms, perhaps the most important step would be to utilize advanced omics and multiomics tools that would leverage the available data to obtain the most informative results through more comprehensive and in-depth analyses. For gene expression data for example, it is well known that biological functions and pathways are regulated by interacting genes, a fact that needs to be taken into consideration during the differential analysis of gene expression data for a more informative interpretation.

Module differential analysis for weighted gene co-expression network, or MODA, is a tool that is able to assemble genes into networks according to their co-expression pattern and additionally informs which sets of co-expressed genes are the most or least regulated by the condition of interest. Network construction is done for both the background count matrix which contains all treatment conditions, as well as for each condition of interest, whose related samples are excluded from the background matrix in order to assess the degree of perturbation and thus, its influence on differential expression. Here, edges are weighted in accordance to the correlation coefficients between each pair of genes. MODA then divides each network into a set of modules containing the co-expressed nodes based on their hierarchical clustering. The segregation of nodes is defined statistically by calculating the average density of the modules then maximizing the optimal cutting height of the dendogram. Following so, MODA proceeds to the differential analysis of the networks, where the Jaccard similarity index is calculated to categorize modules of interests into either condition-specific or conserved, where any conserved module for a condition of interest would have a high Jaccard similarity coefficient with all background modules and would thus be expected to represent general stress response genes.

The main advantage of MODA over other tools of gene expression differential analysis is that it not only looks at differentially expressed genes, but ones that are also co-expressed. Compared to similar network analysis tools like WGCNA, which depends on user input for module assignment, MODA produces the set of modules based on statistical optimization. Furthermore, by segregating both annotated and unannotated genes into modules, MODA can offer insights into the possible functional identities of unknown genes as each module can be expected to contain genes of similar biological functions or pathways.