

Hierarchical cluster analysis as a tool to compare diel time cycles in CAM research

Nathalie Ceuster¹, Stijn Luca², Wim Van den Ende³ en Johan Ceusters^{1,4}

¹KU Leuven, Department of Biosystems, Division of Crop Biotechnics, Research group for Sustainable Crop Production & Protection, Campus Geel, Kleinhoefstraat 4, 2440 Geel, Belgium

² KU Leuven, Department of Electrical Engineering, Kleinhoefstraat 4, 2440, Geel, Belgium

³ KU Leuven, Department of Biology, Laboratory of Molecular Plant Biology, Kasteelpark Arenberg 31, 3001 Leuven, Belgium

⁴ UHasselt, Centre for Environmental Sciences, Environmental Biology, Campus Diepenbeek, Agoralaan Building D, 3590 Diepenbeek, Belgium

Crassulacean acid metabolism (CAM) is an important photosynthetic specialization with optimised water-use efficiency (WUE) by sequestering CO₂ predominantly at night when evapotranspiration rates are low. An important hallmark of CAM plants is the integration of circadian and metabolite control over nocturnal C₄ and daytime C₃ carboxylation processes, hereby providing plasticity for optimizing carbon gain by extending or curtailing the period of net CO₂ uptake over any 24-h period depending on environmental conditions. Inherent to its 'diel origin' the statistical processing of CAM-data is challenging and often limited to comparisons of nocturnal/diurnal accumulations/decreases. As such, specific information in the data might be overlooked, especially when large datasets are considered. To address this issue, 6 months old vegetative *Phalaenopsis* 'Edessa' were sampled over the diel cycle every 2 hours under a light regime of 12h/12h at 28°C and 75% RV (n=5). Besides conventional measurements of malate, soluble sugars and starch, a range of additional metabolites (such as different phosphorylated sugar intermediates, organic acids, polyols, trehalose and trehalose-6-phosphate) were analysed in addition by LC/MS-Q3. These analyses were further complemented by registering diel gas exchange patterns using a LCi Portable Photosynthesis System. As such, a unique CAM dataset arose, consisting of about 2500 data points and representing 38 diel patterns with each diel cycle composed of 13 sampled time points (n=5). In order to maximise data interpretation, an hierarchical agglomerative cluster method was applied after normalization of the data. Clusters will be presented and the physiological and biochemical relevance will be discussed.