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Commentary: Duckweeds as model organisms for metabolic studies

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Duckweeds have many practical applications, for example in human nutrition, as animal feed, in the production of bioplastics or vaccines, and phytoremediation (Acosta et al., 2021). Under most conditions, they reproduce asexually which provides genetically uniform material with predictable patterns of growth that make them ideal as sentinel organisms for phytotoxicity testing (Park et al., 2021). Asexual growth also results in high biomass production which makes duckweeds promising candidates as biofuel feedstocks (Acosta et al., 2021; Liang et al., 2023). In addition, duckweed species like *Lemna minor* and *Spirodela polyrhiza* are also reemerging as model organisms in plant biology as high-quality full genome assemblies and other genomic resources become available (Chang et al., 2016; Acosta et al., 2021). We argue that duckweed species are particularly of interest for the study of primary plant metabolism. Primary metabolism concerns the part of metabolism that is directly involved in the growth and development of plants, and which tends to be highly conserved among plant species. What makes duckweeds particularly attractive is that when grown on liquid media more precise control of physiological conditions can be attained relative to growth of plants in soil. Also, due to their relatively simple anatomical structure and asexual reproduction of fronds by budding, precise characterization of the physiological state under study is possible through one simple metric, *i.e.*, the specific growth rate (rate of dry weight increase per existing dry weight), which can be incorporated relatively easily into metabolic models. This is not possible for land plants, such as Arabidopsis, because over the course of their life cycle, they go through multiple growth stages and phases of anatomical differentiation, which are much more complex to quantify. Furthermore, duckweeds can grow on organic substrates under heterotrophic or photomixotrophic conditions that facilitate isotope tracer studies. For example, in a previous study on duckweed by one of the authors, *Lemna gibba* (L). was grown on glucose with a position-specific ^{13}C-label that can be detected and resolved by Mass Spectrometry or Nuclear Magnetic Resonance spectrometry. Using this approach, the ^{13}C-label was traced into biomass compounds formed from glucose, particularly isoprenoid compounds. Some of the resulting labeling patterns were in apparent disagreement with predictions based on known metabolic pathways for the biosynthesis of isopentenyl pyrophosphate, the universal building block for isoprenoids, (Lichtenthaler et al., 1997). From this data it was deduced that isoprenoid compounds such as carotenoids and isoprenoid chains of phytol and plastoquinone, synthesized in the chloroplast, are produced via a previously unreported plant metabolic pathway, now known as the methylerythitol/deoxyxylulose-5-phosphate pathway (Lichtenthaler et al., 1997).

The principle of tracking individual substrate carbon atoms through metabolic networks is exploited in metabolic flux analysis, a computational approach that provides a quantitative picture of the distribution of metabolic flux through branches and cycles within the primary metabolism network (Sauer, 2006; Clark et al., 2020). Recent studies in plants have shown that such flux measurements in primary metabolism, particularly when combined with other biochemical data such as enzyme activity, metabolomic, transcriptomic or proteomic profiles, can improve our understanding of pathway regulation (Schwender et al., 2015; Canas et al., 2017). Using this idea, we recently set out to demonstrate the usefulness of duckweeds for multi-omic metabolic studies (Shi et al., 2023). *Lemna gibba* fronds were grown under low light on liquid media containing glucose as a carbon source and either nitrate or glutamine added as the nitrogen source. The two nitrogen conditions were then compared by quantification of their growth rates, compositional analyses of frond biomass, targeted metabolomics of about 70 compounds and whole transcriptome analyses. ^{13}C-Metabolic Flux Analysis (^{13}C-MFA) was

carried out, after fronds were grown on ^{13}C-labeled glucose. Regarding growth metrics, when growing on glutamine, *Lemna* grows 1.4 times faster than when fronds grow on nitrate, which is not unexpected since nitrate reduction has an energetic cost whereas glutamine already supplies nitrogen in a reduced form. The results further showed how a reorganization of primary metabolism takes place to accommodate the needs of growing fronds with respect to the different nitrogen inputs. In nitrate grown fronds, all the amino acids of the aspartate and glutamate families are synthesized *de novo* from intermediates of the tricarboxylic acid (TCA) cycle (Figure 1 A). The intermediates withdrawn from the cycle are replaced by organic acids derived from glycolysis. When glutamine is present instead of nitrate, the flux distribution within the TCA cycle changes significantly and is reorganized so that no glycolytic intermediates enter the cycle (Figure 1A, red). Instead, carbon backbones from the abundant glutamine nitrogen source are in part converted into other amino acids of the glutamate family or enter the TCA cycle to be converted into amino acids in the aspartate family. Despite this substantial reorganization of TCA cycle fluxes, we observed that, while well-known nitrate-inducible genes showed expected transcriptional responses, the changes in flux around the TCA cycle were not associated with changes in transcript levels of the corresponding genes. This implies that posttranslational regulation predominates over transcriptional regulation for primary metabolism. In addition to the observations related to the TCA cycle, the ^{13}C-MFA data showed that, while growing with glucose under low light, the Calvin Benson Bassham (CBB) cycle is fully operational and fixing CO_2 (Figure 1B). At the same time, a substantial fraction of glucose carbon is oxidized by passage through the Oxidative Pentose Phosphate Pathway (OPPP), which generates CO_2. The simultaneous activity of CBB and OPPP cycles represent an energy-consuming metabolic cycle, and it is believed that this condition is normally avoided during active photosynthesis by mechanisms that suppress OPPP activity in the light. Recently, however, it appears that the OPPP activity is not always completely inactivated in the light and that certain physiological functions can be attributed to the simultaneous operation of the CBB cycle and OPPP in plants, which is now referred to as the so-called glucose-6-phosphate shunt (Sharkey and Weise, 2016).

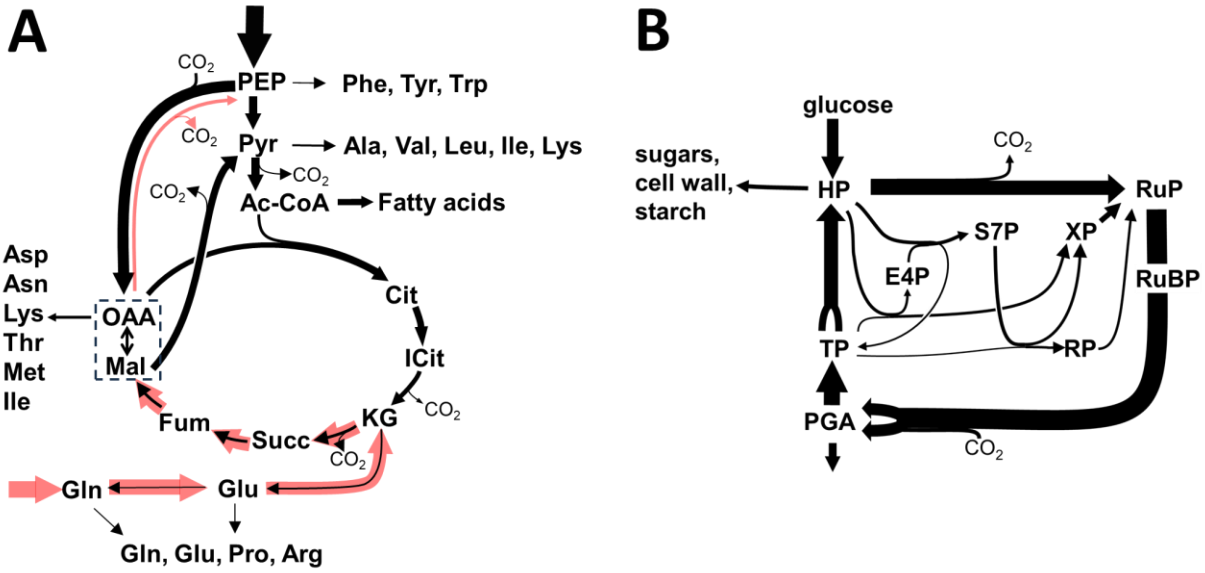

Figure 1: Flux distribution associated to the tricarboxylic acid (TCA) cycle (**A**) and the Calvin-Benson-Bassham (CBB) cycle (**B**) in growing *Lemna gibba* fronds. Arrow thickness represents carbon flux. Black arrows represent the growth condition under which fronds grow on glucose with nitrate as a carbon source. Major changes in flux of significance under the glucose/glutamine growth condition are shown in red. Both panels **A** and **B** are adapted from

Shi *et al.* (2023). Abbreviations: Ac-CoA, Acetyl-Coenzyme A; Cit, Citrate; E4P, Erythrose 4-phosphate; Fum, fumarate; HP, Hexose phosphate; ICit, Isocitrate; KG, α-Ketoglutarate; Mal, Malate; OAA, Oxaloacetate; PEP, Phosphoenol pyruvate; PGA, 3-Phosphoglycerate; Pyr, Pyruvate; RuBP, Ribulose 1,5-bisphosphate; RuP, Ribulose 5-phosphate; S7P, Sedoheptulose 7-phosphate; Succ, Succinate; TP, triose phosphate; XP, Xylulose 5-phosphate.

Taken together, our observations on the reorganization of fluxes around the TCA cycle and operation of the glucose-6-phosphate shunt underscore that primary metabolism is under complex multi-level control and that metabolic flux analysis can play an important role in understanding it. When it comes to engineering primary metabolism of duckweeds, for example to increase accumulation of triacylglycerols to high levels in growing fronds (Liang et al., 2023), metabolic flux analysis can provide important information which along with emerging genetic resources should greatly benefit such engineering and modeling efforts. In recent years, high quality genomes have been published for the duckweed *Spirodela polyrhiza* and 9 other Lemnaceae genome assemblies are currently in publicly accessible sites (Wang et al., 2014; Ernst and Martienssen, 2016; Michael et al., 2017; Hoang et al., 2018; An et al., 2019; Acosta et al., 2021; Ernst and Martienssen, 2023). They should enable detailed comparative analysis of the metabolic architecture across species in the Lemnaceae family and in comparison to other higher plants.

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