Insight

**Systems-level analysis of the plasticity of the maize metabolic network reveals novel hypotheses in the nitrogen-use efficiency of maize roots**

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Alleviating the overuse of nitrogen fertilizer may compromise crop yields, and understanding the efficiency with which plants utilize nitrogen will help us engineer crops to maintain yields while using less fertilizer. As the assimilated nitrogen is necessary for the biosynthesis of many components of plant biomass, there is interest in studying the nitrogen-use efficiency (NUE) of crops in a systemic manner. In their article in this issue of *JXB*, Chowdhury et al. (2022) analyze the behavior of the metabolic network in the maize root as it responds to growth in nitrogen-poor conditions. The authors reconstructed the metabolic network of the maize roots *in silico*, enabling them to integrate both transcriptomics and metabolomics data sampled from roots of maize seedlings grown under either nitrogen-rich or nitrogen-deficient conditions. Crucially, they run simulations on the metabolic network using the integrated data to constrain the outcomes, and examine how reaction fluxes and metabolite pools vary between the two conditions.

**Nitrogen deficiency in maize**

The demand for a chemical source of nitrogen as a fertilizer for all the world’s crops is unsustainable, partly due to the environmental impact of using fossil fuels to drive the synthesis of fertilizer and partly due to the negative effect of dumping large quantities of fertilizer on native ecosystems (Udvardi et al. 2015; Wang et al., 2021). Reducing the amount of fertilizer while maintaining the yield that would feed the world’s population has been an important goal and there is burgeoning research into the efficiency with which crops utilize nitrogen (Li et al., 2017; Kant et al., 2011). It was estimated that a 20% increase in NUE would reduce the nitrogen requirements by 1.4 Mt year⁻¹ (Langholtz et al., 2021). In order to examine the relationship between the use of fertilizer and the yield of the crops, researchers are growing crops in nitrogen-poor conditions and examining the mechanisms by which nitrogen is assimilated (Amiour et al., 2012). The responses are not only observed in the transporters and subsystems that assimilate N directly; they ripple across the primary metabolic processes that produce biomass as part of the plant’s development and growth, impacting the eventual yield, and highlighting the need for a system-level phenotype to characterize them (Kant et al., 2011).

**Genome-scale reconstruction of maize tissue**

The reconstruction and simulation of the entire metabolic network encoded in the genome of any species has been an important tool in understanding the systemic manner in which an individual may respond to perturbations in its environment. The reconstructions are the result of the extensive collating of enzymes and the biochemical reactions they catalyze from the available data in databases and the literature. Genome-scale metabolic reconstructions for plants have been built and rebuilt since the first published models of the core central carbon networks for *Arabidopsis thaliana* (Poolman et al., 2009) and *Chlamydomonas reinhardtii* (Boyle and Morgan,
Metabolic reprogramming is a systemic response

The reconstruction of the genome-scale metabolic network of the maize root in silico allowed the authors to examine the systemic impact of the nitrogen-limiting growth regime on the root tissue. Some of the same authors previously published a similar study on the NUE in the maize leaf, with the data being sampled from several different developmental stages during the plant’s growth (Simons et al., 2014), and the authors are now focused on performing some of the same analyses in the maize root. Much of this work is focused on the manner in which the network is built, is simulated using the integrated data, and how the approach is validated by comparing the experimental pooling of metabolites with their theoretical results. One novel approach that the authors take here is to extend the flux-sum analysis approach (Chung and Lee, 2009; Lakshmanan et al., 2015) to simulate a proxy for the range of concentrations at which a metabolite may pool under the environmental conditions, which they call flux-sum variability. By using this approach, they highlight eight lipids, and two precursors (methionine and citicoline), whose metabolic pool sizes were not directly coupled to the biosynthesis of biomass components, and hypothesize that they play a role in the increased growth of roots that occurs under nitrogen-poor conditions.

Systems-level responses extend beyond tissues

The metabolic network that was reconstructed for any tissue can be combined with those reconstructed for other tissues and organs in the same species. As the authors have already generated the metabolic network for the maize leaf, a natural step forward in this work would be to connect the networks representing the root and leaf tissues, via the means of a metabolic network representing stem tissue, so as to combine the sources of assimilated nitrogen and carbon, respectively. Whole-scale plant tissue modeling has already been implemented and advocated by several groups (Grafahrend-Belau et al., 2013; Shaw and Cheung, 2018; Gomes de Oliveira Dal’Molin et al., 2015). For the individual tissues, these sources are modeled as fixed inputs from the ‘environment’, either sucrose for the root or amino acids for the leaf, but by combining the two metabolic networks for each tissue and allowing the abundance of these metabolites to be dynamically constrained by the integration of omics data, researchers may reach additional conclusions on how the mechanisms of nitrogen assimilation and carbon assimilation may interact.

Are there key metabolic shifts during development?

The conclusions in this work were reached using data that represent a single snapshot during the development of maize seedlings. For the purpose of the study, half of the maize seedlings were grown in nitrogen-poor conditions until sampled at the 6- to 7-leaf stage and, as such, the reprogramming in question had occurred over the course of two and a half weeks. While the catalysis of the enzymes in the network occurs within very short time scales, the spatial and temporal pooling of key metabolites can occur over longer time scales. The pooling of these metabolites may in turn impact the regulation of the metabolic network via several means, but could also do so unevenly, where different metabolites pool at various rates, affecting metabolic subsystems at different stages of the plant’s development. Large-scale sampling of these metabolites at these developmental stages may reveal the timing of these metabolic shifts, but the generation of such datasets is expensive and time-consuming, particularly as the data are noisy, and the researchers may not know in advance when to sample the tissues. The work performed here could be done in combination with other approaches such as dynamic flux balance analysis (Mahadevan et al., 2002; Flassig et al., 2016; Shaw and Cheung, 2018; Schroeder and Saha, 2020) in order to guide this approach, to discover when these metabolic shifts occur, and improve the temporal targeting of the engineering for NUE.

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References

Amiour N, Imbaud S, Clément G, et al. 2012. The use of metabolomics integrated with transcriptomic and proteomic studies for identifying key steps involved in the control of nitrogen metabolism in crops such as maize. Journal of Experimental Botany 63, 5017–5033.

Boyle NR, Morgan JA. 2009. Flux balance analysis of primary metabolism in Chlamydomonas reinhardtii. BMC Systems Biology 3, 4.

Chowdhury NB, Schroeder WL, Sarkar D, Amiour N, Quilleré I, Hirel B, Maranas CD, Saha R. 2022. Dissecting the metabolic reprogramming of maize root under nitrogen-deficient stress conditions. Journal of Experimental Botany 73, 275–291.

Chung BK, Lee DY. 2009. Flux-sum analysis: a metabolite-centric approach for understanding the metabolic network. BMC Systems Biology 3, 117.

Flassig RJ, Fachet M, Höfler K, Barton PI, Sundmacher K. 2016. Dynamic flux balance modeling to increase the production of high-value compounds in green microalgae. Biotechnology for Biofuels 9, 165.

Gomes de Oliveira Dal’Molin C, Quek LE, Saa PA, Nielsen LK. 2015. A multi-tissue genome-scale metabolic modeling framework for the analysis of whole plant systems. Frontiers in Plant Science 6, 4.

Grafahrend-Belau E, Junker A, Eschenröder A, Müller J, Schreiber F, Junker BH. 2013. Multiscale metabolic modeling: dynamic flux balance analysis on a whole-plant scale. Plant Physiology 163, 637–647.

Kant S, Bi Y-M, Rothstein SJ. 2011. Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. Journal of Experimental Botany 62, 1499–1509.

Lakshmanan M, Kim TY, Chung BK, Lee SY, Lee DY. 2015. Flux-sum analysis identifies metabolite targets for strain improvement. BMC Systems Biology 9, 73.

Langholtz M, Davison BH, Jager HI, Eaton L, Baskaran LM, Davis M, Brandt GC. 2021. Increased nitrogen use efficiency in crop production can provide economic and environmental benefits. The Science of the Total Environment 758, 143602.

Li H, Hu B, Chu C. 2017. Nitrogen use efficiency in crops: lessons from Arabidopsis and rice. Journal of Experimental Botany 68, 2477–2488.

Machado D, Herrgård M. 2014. Systematic evaluation of methods for integration of transcriptomic data into constraint-based models of metabolism. PLoS Computational Biology 10, e1003580.

Mahadevan R, Edwards Jeremy S, Doyle FJ 3rd. 2002. Dynamic flux balance analysis of diauxic growth in Escherichia coli. Biophysical Journal 83, 1331–1340.

Poolman MG, Miguel L, Sweetlove LJ, Fell DA. 2009. A genome-scale metabolic model of Arabidopsis and some of its properties. Plant Physiology 151, 1570–1581.

Schroeder WL, Saha R. 2020. Introducing an optimization- and explicit Runge-Kutta-based approach to perform dynamic flux balance analysis. Scientific Reports 10, 9241.

Seaver SM, Bradbury LM, Frelin O, Zarecki R, Ruppin E, Hanson AD, Henry CS. 2015. Improved evidence-based genome-scale metabolic models for maize leaf, embryo, and endosperm. Frontiers in Plant Science 6, 142.

Shaw R, Cheung CYM. 2018. A dynamic multi-tissue flux balance model captures carbon and nitrogen metabolism and optimal resource partitioning during Arabidopsis growth. Frontiers in Plant Science 9, 884.

Simons M, Saha R, Amiour N, et al. 2014. Assessing the metabolic impact of nitrogen availability using a compartmentalized maize leaf genome-scale model. Plant Physiology 166, 1659–1674.

Tong H, Küken A, Razaghi-Moghadam Z, Nikoski Z. 2021. Characterization of effects of genetic variants via genome-scale metabolic modelling. Cellular and Molecular Life Sciences 78, 5123–5138.

Udvardi M, Brodie EL, Riley W, Kaeppler S, Lynch J. 2015. Impacts of agricultural nitrogen on the environment and strategies to reduce these impacts. Procedia Environmental Sciences 29, 303.

Wang F, Yoshida H, Matsuoka M. 2021. Making the “Green Revolution” truly green: improving crop nitrogen use efficiency. Plant & Cell Physiology 62, 942–947.