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Sowing the seeds for advanced synthetic plant biology

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Constructing synthetic gene circuits has always been at the heart of synthetic biology. Various examples for bacteria, yeast and mammalian cells have been demonstrated (1–4) and enabled applications in metabolic engineering (5), therapeutic cells (6) and biosensing (7). However, genetic circuits are still at an early stage in plants and especially more complex logical operations have not been shown yet (8, 9). Recently, Brophy *et al.* established a rapid workflow for prototyping genetic circuits in tobacco leaves and subsequently used the learned design principles to control spatial gene expression patterns in the root tip of *Arabidopsis thaliana* and to alter the root architecture (10). The ability to exactly control where in a plant and when genes are expressed could open up the possibility in the future to engineer crops to respond to extreme conditions, such as drought, extreme heat and flooding (11).

The plant engineering toolkit developed by the Dinneny laboratory at Stanford University that was recently published in Science presents a major advancement for the field of plant synthetic biology, in both scope and complexity. Plants grow slow. This makes it difficult to go through multiple rounds of the design–build–test–learn cycle or test multiple designs in parallel, which is typically required to engineer functional circuits. Furthermore, the engineering of complex multicellular organisms has remained almost unexplored, as genetic parts, which have been characterized to operate on the whole organism level, have been missing. To overcome these hurdles, the authors started by constructing the basic building blocks for their genetic circuits, which are synthetic transcription activators and repressors. Developing these tools necessitated several rounds of testing and debugging, and the authors not only describe those designs that worked but also commendably describe failed designs. By using transient expression in tobacco leaves as an intermediate model system, they were able to technically overcome the slow-growth constraint in plants and could measure the performance of a circuit within 2 days instead of waiting several weeks for stably transformed plants. This was possible by co-infiltrating the leaves with *Agrobacterium* strains—a bacterium that has the natural ability to genetically transform plants—containing the different combinations of the circuit inputs (which are encoded on separate plasmids) and outputs. Depending on which combination of plasmids had been infiltrated, different circuit states could be obtained.

After prototyping via their transient assays in tobacco leaves, the authors moved to stable transformed *Arabidopsis* lines, where root tips were used as a model for spatial-specific gene circuits—circuits that control at which position in a plant genes are expressed. Although many findings could be transferred from the prototyping phase in tobacco, several logical operators differed and needed further adjustments and debugging. After this tuning phase, all logic gates resulted in the desired expression patterns and the authors continued by utilizing these synthetic gene circuits to engineer root branching—a process that allows a plant to expand their root system to ensure water and nutrient supply. At the end of this tedious endeavor of designing, building and testing, the authors could successfully show that their developed tools can be used for highly fine-tuned spatial gene expression patterns, which enable the engineering of complex processes, such as regulation of the root architecture.

In the future, the platform and the tools developed in this work are not limited to engineering plant morphology, but could also be utilized for more ambitious plant engineering efforts such as the C4 Rice project, where one of the limitations is to achieve spatial-specific gene expression for at least 20 or more genes (12). This work demonstrates the first step to moving from single cells to more complex multicellular organisms and also provides a starting point to engineer other multicellular chassis, such as animal models.

Even though many challenges for the field still lay ahead, the tools developed in this work represent a very important milestone for plant synthetic biology and could in the future significantly contribute to engineering the next generation of crops that are able to cope with the effects of the climate crisis.

Conflict of interest statement. None declared.

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