Executive Summary

Overcoming Barriers in Plant Transformation

A Focus on Bioenergy Crops

U.S. DEPARTMENT OF ENERGY | Office of Science

Biological and Environmental Research Program

Overcoming Barriers in Plant Transformation: A Focus on Bioenergy Crops

Virtual Workshop

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Office of Science
Biological and Environmental Research Program

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About BER

The Biological and Environmental Research (BER) program supports transformative science and scientific user facilities examining complex biological, Earth, and environmental systems for clean energy and climate innovation. BER research seeks to understand the fundamental biological, biogeochemical, and physical principles needed to predict a continuum of processes occurring across scales, from molecules and genomes at the smallest scales to environmental and Earth system change at the largest scales. This research—conducted at universities, U.S. Department of Energy national laboratories, and research institutions across the country—is contributing to a future of reliable, resilient energy sources and evidence-based climate solutions.

This executive summary is available at genomicscience.energy.gov/plant-transformation/

Full report coming spring 2024.

Executive Summary

Although 2023 marks the 40th anniversary of the first transgenic plant, routine transformation of most plant genotypes remains elusive. Rapid systems to overexpress, interfere, or knock out genes—collectively defined in this report as "transformation and editing technologies"—are needed to understand plant gene function. This understanding in turn is crucial for efficiently developing new, sustainable, high-yielding, and climate-resilient crops to meet the growing demand for food, feed, fiber, and fuel. In particular, the ability to apply transformation and editing technologies to bioenergy crops has remained largely unrealized. To address this opportunity, the U.S. Department of Energy (DOE) Biological and Environmental Research Program convened a workshop on September 18–20, 2023, to define transformation and editing needs and barriers focused on bioenergy crops. The main conclusions are summarized below.

Community Needs for Plant Transformation Now and in the Future

The gap between transformation capacity and need was a common theme during the workshop. Participants universally described today's need for faster, cheaper systems that are more genotype-flexible. In the next 5 to 10 years, transformation demand is expected to increase at least 20-fold, and more sophisticated genomic engineering will require efficiency increases of at least one order of magnitude. The metabolic engineering and synthetic biology needed to address the burgeoning bioeconomy will also require the ability to introduce long DNA sequences containing tens of genes. Therefore, genotype-flexible, high-throughput, and fast-transformation systems are increasingly and urgently needed.

Current State and Challenges of Plant Transformation

Most DOE-relevant bioenergy crops present unique challenges for transformation compared to food crops in that they are long-lived perennials and obligate outcrossers. For many bioenergy crops, germplasm can only be maintained as living plants, thus requiring additional plant growth capacity and new methods for maintenance and preservation. A disproportionate number of these bioenergy crops are monocots, which are generally less amenable (i.e., recalcitrant) to *Agrobacterium*-mediated transformation (see Fig. ES.1, p. 2). Many are polyploid, meaning their genomes contain highly duplicated genes, thus requiring efficient multiplexed editing. In addition to increased capacity for bioenergy crop transformation, advanced transformation technologies are needed to enable and expedite synthetic biology research. Such advances include tissue- and cell-type-specific promoters, efficient linkers to generate multigenic constructs, and ways to integrate large DNA constructs into plant genomes. Development of landing pads or similar technologies also is needed to obtain site-specific insertion of constructs.

Current plant transformation facilities tend to specialize in only a few crops, require subsidies, and must balance their efforts between producing transgenic or edited plants and negotiating contracts and intellectual property (IP). Few have the time or resources for research to improve methodologies or efficiencies, and all compete with the private sector for personnel. Because methods can be very species- and genotype-specific, specializing in a broad array of crops is challenging for any one center. In the future, a coordinated network of transformation centers, each with its own crop specialties, may better accommodate transformation needs. However, transformation centers alone will not solve current transformation limitations. Also needed are (1) additional research into tissue culture and regeneration biology, (2) development of new technologies, (3) development and incorporation of automation and artificial intelligence (AI), and (4) training of a workforce skilled in tissue culture and transformation technologies.

New Methods for Gene Delivery, Transformation, and Regeneration

The biology and genetic mechanisms underlying plant regeneration are not well understood. Deepening the

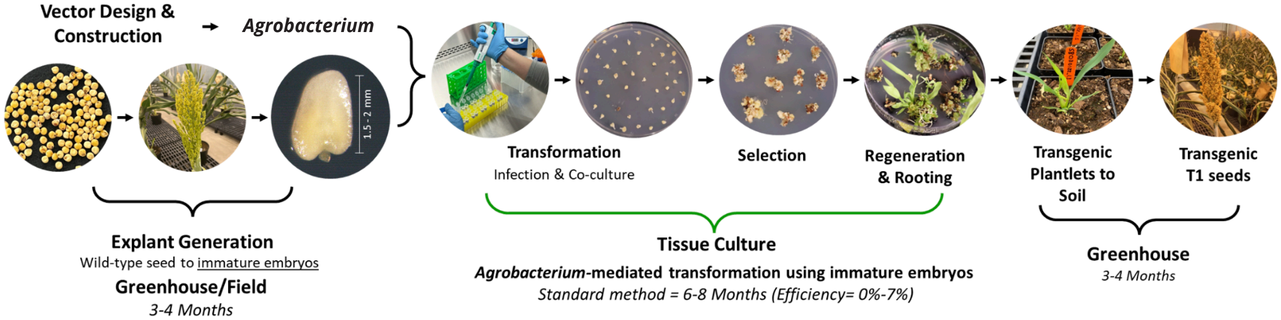

Fig. ES.1. Standard *Agrobacterium*-Mediated Plant Transformation Workflow Exemplified Using Sorghum Bicolor. Shown left to right are representations of various steps and time (months) required for each phase beginning with the generation of explants (i.e., immature embryos) from panicles of the donor plant. Next is the tissue-culture phase during which transformation, selection, regeneration, and rooting are initiated. Finally, in the greenhouse phase, putative transgenic plants are transplanted in soil and then mature T1 seeds are harvested. Various steps in standard tissue-culture workflows are time-consuming and labor-intensive. Each step requires extensive hands-on experience in the fields of plant transformation and cell biology, including access to quality infrastructure. Recent advancements in transformation technologies, such as the utilization of morphogenic regulator genes, have significantly improved transformation efficiency by tenfold, and timelines in the tissue-culture phase are reduced from 6 to 8 months to 1.5 to 2 months while offering genotype flexibility to transform highly recalcitrant economically important crops.

understanding of these mechanisms, including somatic embryogenesis and DNA repair, requires short- and long-term strategies to increase editing and transformation efficiencies, including studies on the molecular basis of recalcitrance. With appropriate research investment, several incipient technologies could become viable and facilitate regeneration, transformation, and editing across a wide gamut of bioenergy species. The discovery of additional growth-regulator genes could improve regeneration. Transformation and editing could be improved by (1) developing tissue-culture-free systems; (2) using viral delivery of sgRNAs and Cas nucleases; (3) perfecting nanoparticles for reagent delivery; and (4) developing improved strains of *Agrobacterium*, artificial chromosome technology, and tunable or synthetic promoters. Advances in robotics, coupled with AI, could revolutionize tissue culture.

Leveraging Omics Approaches to Develop Future Transformation Technologies

New genomic tools enable the generation of extremely accurate genome sequences that precisely define polyploid bioenergy crop gene variants that have diverged over time. However, in these crops, little is known about gene function, promoters, and regulatory elements for these polyploid genes. Comprehensive genomic resources thus are needed to facilitate the development of next-generation transformation technologies relevant to bioenergy crops. Information from assays such as metabolomics, proteomics, single-cell transcriptome atlases, transposase-accessible chromatin with sequencing (ATAC-seq), and DNA affinity purification sequencing (DAP-seq) will accelerate improvements in transformation and targeting technologies.

Intellectual Property, Regulatory Landscape, and Stewardship

Transformation facility personnel and researchers alike need to be familiar with the issues associated with IP and how these issues impact the ability to conduct research. IP can apply to genes, vectors, methods, processes, and even the plant variety being transformed or edited.

The U.S. Department of Agriculture (USDA), U.S. Food and Drug Administration, and U.S. Environmental Protection Agency (EPA) are the primary federal agencies that regulate transgenic plants. Of these, USDA regulations affect laboratory and field-level research the most. If EPA decides to regulate and require pesticide registration for many of the growth alterations being considered for bioengineered bioenergy crops, this would greatly impede their development and deployment (**EPA 2023**; **EPA 1994**).

Finally, for stewardship, every facility should have an established set of standard operating procedures to minimize the possibility of unintentional release of edited or transgenic reproductive propagules.

Developing an Inclusive Community and Talent Pool

Transformation and editing laboratories require staff with multiple levels of training, ranging from people well-versed in the theory and biology of transformation and regeneration to those skilled in laboratory work. A concerted effort will be necessary to provide the research community with role models, needed curricula, and training opportunities. PhD programs, internships, apprenticeships, and micro-courses are practical and effective solutions. Community colleges can be particularly effective for tapping into groups historically underrepresented in plant transformation science.

Plant Transformation Needs and Opportunities

- A DOE research laboratory that performs long-term cutting-edge research on the transformation of bioenergy crops at a scale beyond the capacities of existing academic research laboratories in terms of both cost and duration.
- A coordinated network of DOE-funded plant transformation facilities, each of which specializes in a subset of bioenergy crops. These facilities would provide state-of-the-art transformation services and resources to meet the growing demand for transformation capacity of the DOE and academic researcher community.
- Funding and training to develop a diverse workforce in plant transformation and provide opportunities to attract and retain these skilled researchers for the long term.
- DOE competitive funding opportunities for the community to perform basic research on transformation and regeneration biology and methodology.