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# 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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This report is available at genomicscience.energy.gov/plant-transformation/

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# Overcoming Barriers in Plant Transformation: A Focus on Bioenergy Crops

## Workshop Report

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# Contents

# Executive Summary

Although 2023 marked 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. vi). 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 is also needed to obtain site-specific construct insertion.

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. [Courtesy Veena Veena, Donald Danforth Plant Transformation Facility]

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 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 the 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 (U.S. EPA 2023; U.S. 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 that have been 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.

# Chapter 1
# Introduction

The first transgenic plant was produced over 40 years ago. Since then, almost all commercially important plants have been transformed at least once, and transgenic hybrids and cultivars predominate in corn, canola, soybean, and cotton. Yet, routine transformation of most plant genotypes remains elusive. For the most part, only one or few genotypes within a crop are amenable to transformation and editing (see Glossary, p. 34, for definitions of these and other terms).

Parallel advances in genomics and gene editing have enabled the use of functional genomics to study plant gene function. Understanding plant gene function is crucial to efficiently develop the new, sustainable, high-yielding, climate-resilient crop varieties required to meet growing demands for food, feed, fiber, and fuel. Consequently, the need to understand plant gene function has dramatically increased the need for transformation; however, available methodologies for plant transformation and gene editing are too inefficient to meet this demand.

## Plant Transformation Recognized as a Growing Priority

The various limitations preventing increased efficiency were discussed in a 2015 National Science Foundation (NSF) workshop on plant transformation in Clearwater, FL. Limitations and innovations to overcome them were further discussed in a subsequent white paper (Altpeter et al. 2016). In the years since the NSF workshop, research efforts to improve the ability and capacity for plant transformation are materializing. For example, NSF released a Dear Colleague Letter on advancing plant transformation for crops (NSF 2022), and the U.S. Department of Agriculture (USDA) listed plant tissue culture and editing as priority areas in its Science and Research Strategy plan for 2023–2026 (USDA 2023). However, until now, the discussion has centered on food, feed, and fiber crops, while the ability to apply these 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. Technology experts, service providers, users, and stakeholders attended presentations and breakout sessions focusing on several objectives:

- Evaluating current and future transformation needs.
- Assessing the current capacity for transformation.
- Identifying transformation and editing challenges.
- Considering how emerging technologies can be leveraged to facilitate work on bioenergy crops.
- Surveying the intellectual property (IP) and regulatory landscape.
- Defining the requirements and need for a trained workforce.

This report summarizes the workshop's main conclusions and identifies research needs and opportunities for the future of bioenergy crop transformation.

## Community Needs Continue to Increase

The gap between current transformation capacity and transformation demand in the U.S. is a common theme across bioenergy crops—and across crops in general. U.S. laboratories are collectively transforming an estimated 5,000 to 10,000 constructs per year into crop species—with 6 to 10 quality events needed per construct. However, bioenergy crops presently account for just about 200 constructs per year. At this rate, current transformation capacity will not meet the growing demand for bioenergy crop transformation,

which is expected to increase 10-fold per year. Moreover, across plant species and genotypes, transformation and genome editing face common limitations at almost every stage. The sections below outline these limitations and discuss future needs to help close the gap between transformation capacity and demand through faster, cheaper transformation systems that are more genotype-flexible.

## Limits to Progress

Plant transformation and editing occur at the single-cell level and involve two critical aspects: introducing the needed reagents into the cell (i.e., transformation) and recovering a whole plant from that one cell (i.e., regeneration). Most plant genotypes are recalcitrant to regeneration and/or transformation (i.e., the crop lacks genotype flexibility). Hence, transformations are performed in only a few genotypes within any given species. Unfortunately, these genotypes are seldom those of greatest utility to researchers, growers, or breeders. Finally, as transformation and regeneration are labor-intensive processes, lack of technology automation confounds the challenge.

Lack of genotype flexibility underlies common limitations that affect all crops:

- Costs of generating transgenic or edited events remain too high relative to available research dollars.
- Service providers are unable to meet transformation and editing needs without subsidies.
- Transformed or edited plants ready for phenotyping take significant time to obtain, with time frames often extending beyond the typical 3-year federal funding cycle.
- Effective screening for desired phenotypes requires many transgenic and edited events, which exacerbates the transformation capacity shortage.

## Improved Screening and Editing Efficiency Are Needed

Low transformation and regeneration efficiency rates not only exacerbate the scope of molecular and phenotypic screening needed to confirm an edited event, but also hinder development of novel methods for efficient editing without integrating DNA into the plant genome.

Historically, efforts to understand how a transgene impacts phenotype have been confounded by the variability associated with random integration. As a result, finding optimal transgenic events, or events that are of high quality,[1] has required screening tens to hundreds of transgenic events. Recombinase-mediated or Cas-mediated gene targeting technology confers the ability to recover primarily single-copy insertion events and would reduce the current requirement to generate transgenic plants in large numbers. Site-specific integration of a transgene into a predetermined location reduces expression variability to the point that only a few transgenic events are required to accurately assess expression (Gao et al. 2020a). Hence, recombinase-mediated or Cas-mediated gene targeting technology could dramatically reduce the trait characterization pipeline for all crops in transformation laboratories.

However, developing such technology for the academic community will require substantial investment to (1) improve infrastructure (e.g., laboratories, growth chambers, and greenhouse facilities), (2) train skilled staff, (3) establish a requisite knowledgebase, and (4) enable access to methodologies and biomaterials. Most academic facilities were established long ago to meet organizational needs that anticipated limited use by a few labs for a few crops; consequently, many have older infrastructure and equipment. Exacerbating the issues faced by aging facilities are the recent success and potential benefits of CRISPR/Cas-mediated genome modification, which further expand the gap between current methodology and the community's needs. Indeed, CRISPR/Cas technology can only be expected to become more versatile and useful, which will increase transformation and editing demands even further.

In addition, advanced genomic resources and computational methods will increase transformation demand at least 20-fold in the next 5 to 10 years, and more

[1] In random integration, quality refers to engineered plants that have only one copy of the intact transgene in their genome and in a location that does not affect other genes. For CRISPR-based approaches, quality refers to generation of the desired edit(s) and production of transgene-free progeny.

sophisticated types of nuclease-mediated genomic engineering will necessitate efficiency increases of at least one additional order of magnitude. Meeting these demands will require a platform to rapidly screen even higher numbers of T0 plants to identify desired outcomes of using either homology-directed repair (HDR) or nonhomologous end joining (NHEJ) pathways to repair double-strand DNA breaks.

Furthermore, 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—at least 30 genes at once, according to some estimates. Therefore, high-throughput, fast-transformation, and regeneration systems are urgently and increasingly needed. In an ideal scenario, these more advanced systems could even eliminate labor-intensive and time-consuming tissue culture altogether.

Chapter 2

# Current State and Challenges of Plant Transformation

Plant transformation and editing has advanced significantly over the past 40 years. However, limitations of current transformation methods prevent increased efficiency, and existing plant transformation facilities are ill-equipped to meet the growing demand for increased transformation needs, especially for bioenergy crops. This chapter outlines the current state and challenges of bioenergy crops and bioenergy crop facilities and identifies potential opportunities to improve transformation capacity and enhance transformation centers.

## Bioenergy Crops

Currently, plant transformation within and beyond bioenergy species uses three main categories of DNA delivery: (1) *Agrobacterium*; (2) viruses; and (3) physical methods, such as particle gun, protoplast transfection, and nanoparticles. Of these, *Agrobacterium*-mediated transformation and particle gun-mediated transformation have been mainstream for decades. While each of these methods have specific strengths, they also pose challenges that must be addressed to successfully increase capacity for bioenergy crop transformation. Additionally, more advanced transformation technologies are needed to enable and expedite research. During the workshop, participants identified potential universal and crop-specific needs that could serve as a starting points for developing a more comprehensive community solution across crops.

### *Assessing Current DNA Delivery Methods for Transformation*

With particle bombardment, DNA can be delivered across all bioenergy crops and a wide variety of explants by adjusting parameters during the transformation protocol. However, this method sometimes results in a higher copy number of integrated DNA fragments if too much DNA is delivered (Altpeter et al. 2016). Collateral damage in the form of genomic fragmentation and rearrangement also can occur (Svitashev et al. 2002). Multicopy integrations and the accompanying collateral genomic damage could have a detrimental effect on the plant, and thus should be avoided, particularly in clonally propagated crops where they cannot be segregated away from a transgene or edit. In comparison, *Agrobacterium*-mediated transformation works well in many species, and typically results in low copy transgene integration and fewer changes to surrounding genomic sequences. However, species and explant combinations can be recalcitrant to *Agrobacterium* infection. Challenges must be solved for both methods to (1) continue expanding the plant host range and tissue types for *Agrobacterium* and (2) adapt both methods to be more effective at meristem transformation.

For the near future, *Agrobacterium*-mediated delivery likely will be the main path forward for bioenergy crops. Additionally, recent publications on use of RNA viruses for virus-mediated delivery have demonstrated promising results, specifically for transient delivery of guide RNAs (gRNAs) for editing (Ellison et al. 2020). Other technologies such as the delivery of mobile RNAs through grafting (e.g., in *Arabidopsis*) that allow the movement of both Cas9 RNA and gRNAs from transgenic roots into nontransgenic shoots for successful editing (Yang, L., et al. 2023) also hold promise. Such methods will continue to evolve.

### *Advancing Transformation Technologies*

Transformation objectives are evolving beyond the single trait approach that has been predominant since the early 1980s when transformation was adopted as a tool. Presently, 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. Particular attention needs to be placed on using landing pads to obtain site-specific construct insertion.

As plant research moves from simple transgenics (i.e., single-gene) to multigene transformation (i.e., multiple genes for pathway engineering or for trait stacking), or from simple CRISPR/Cas-mediated mutagenesis to multiplexed mutagenesis, cisgenic, intragenic, or even more complex genome engineering, the number of transgenic events needed for quality molecular event screening is likely to increase some 10- to 200-fold depending on the desired outcome. For example, next-generation genome modifications using Cas-mediated homology-dependent repair (HDR) require transformation frequencies approximately 100-fold higher than those needed for simple mutagenesis (Huang and Puchta 2019; Svitashev et al. 2015).

Most DOE-relevant bioenergy crops also present unique challenges to plant transformation in that they are long-lived perennials and obligate outcrossers. As they do not breed true from seeds, specific genotypes can only be maintained as living plants, thus requiring additional plant growth capacity and new methods for maintenance and preservation. The outcrossing nature of many bioenergy crops poses further complications for functional genomics in that no two plants in the same variety have the same genotype, and these genetic differences can confound functional genomic studies. Bypassing this problem necessitates the use of selected genotypes, which in turn requires the use of cell lines started from mature plants. However, starting cell lines from mature plants is usually more difficult—if possible at all—than starting cell lines from embryos or seeds. Finally, many bioenergy crops are polyploid, in which the genome contains large numbers of paralogs. Thus, effective knockout of a gene implies knocking out all the homoeologous copies and their paralogs, which means that efficient multiplexed editing is a necessity.

## *Using Potential Crop-Specific Needs for a Comprehensive Community Solution*

During the workshop, participants discussed potential crop-specific needs of bioenergy species (see Fig. 2.1, p. 6), model plants, and food crops. These needs are not necessarily drivers for a more comprehensive community solution across crops. However, they represent important points that could serve as a starting point for considering such a solution. For all vegetatively propagated species, the difficulty to segregate a transgene makes the development of nonintegrative methods for genome modification essential.

### Bioenergy Species

#### *Camelina*

Camelina is a relative of *Arabidopsis*. As such, several genotypes can be transformed via floral dip (Liu et al. 2012; Sitther et al. 2018). Camelina is the only bioenergy crop that may currently have the transformation efficiency to permit the generation of HDR events in a dedicated laboratory. Nevertheless, as with *Arabidopsis* (Zipfel et al. 2006), the use of modified *Agrobacterium* strains that elude plant immune recognition may increase the efficiency transformation in camelina (Yang, F., et al. 2023).

#### *Miscanthus*

Gene editing of the diploid and tetraploid *M. sacchariflorus*, diploid *M. sinensis*, along with their commonly planted sterile triploid hybrid, *M. x giganteus*, has been recently demonstrated. *Miscanthus* does not breed true from seed, so all stable edited material must be propagated through rhizomes (Trieu et al. 2022).

#### *Poplar*

Standard transformation methods for poplar are only well-established for one clone, Inra 717-1B4, an interspecific hybrid (Mader et al. 2017). Other genotypes remain difficult to transform (Sulis et al. 2023). In addition, generating transgenic plantlets ready for soil still takes over one year. Much higher throughput and genotype flexibility are required to take advantage of recent advances in gene and trait discovery.

#### *Sorghum*

As is often the case with grasses, when traditional technology is used sorghum transformation depends

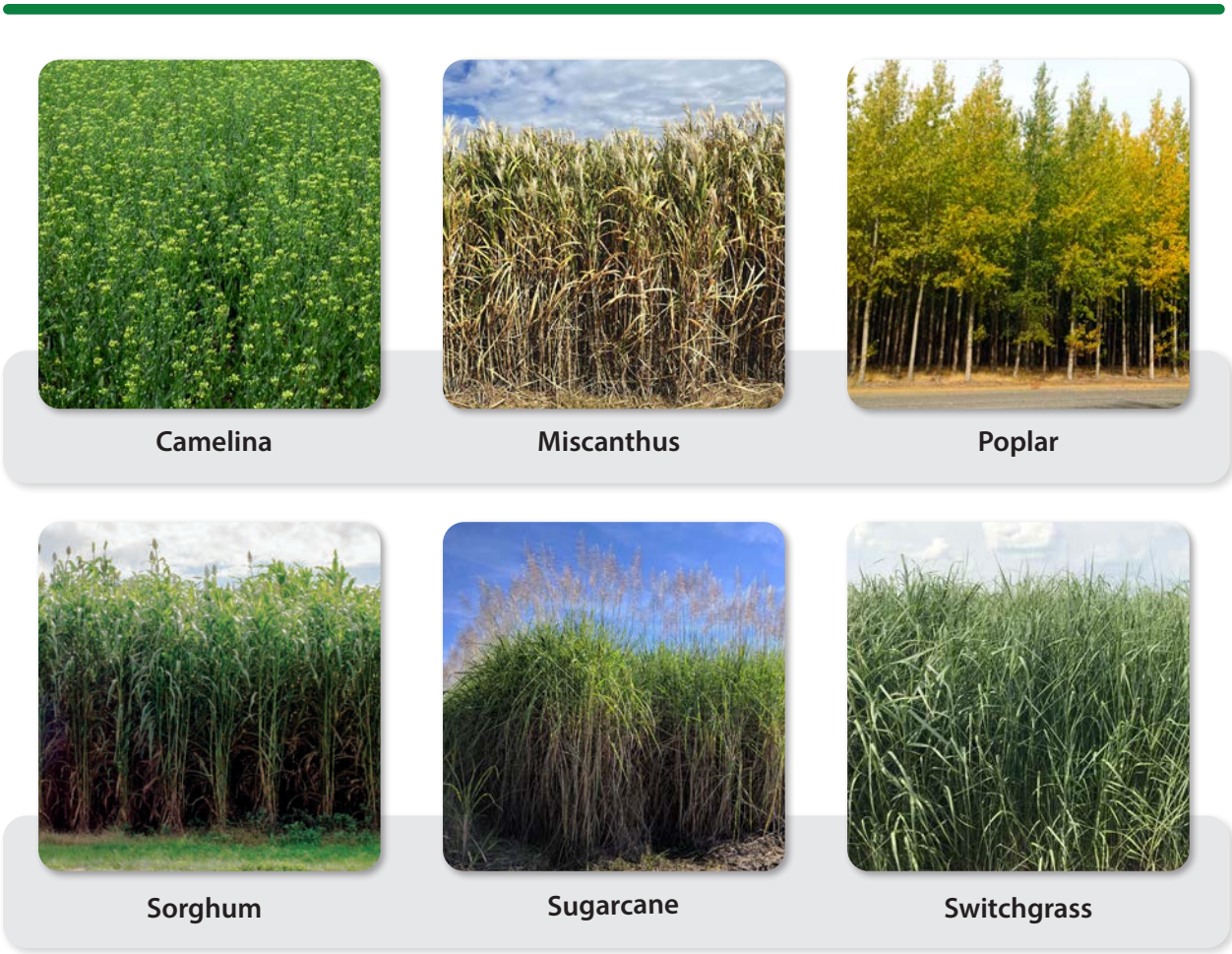



**Fig. 2.1. Enhancing Bioenergy Crop Transformation.** The ability to apply transformation and editing technologies to bioenergy crops has remained largely unrealized. Most DOE-relevant bioenergy crops also present unique challenges to plant transformation. Identifying potential crop-specific needs of bioenergy species, along with those of model plants and food crops could serve as a starting point for developing a more comprehensive community solution for advancing transformation and editing technologies across crops. [Camelina courtesy Michigan State University. Miscanthus courtesy Jeremy Schmutz, HudsonAlpha Institute for Biotechnology. Poplar courtesy Oak Ridge National Laboratory. Sorghum courtesy Center for Advanced Bioenergy and Bioproducts Innovation. Sugarcane courtesy Brandon James, HudsonAlpha Institute for Biotechnology. Switchgrass courtesy Jeremy Schmutz, HudsonAlpha Institute for Biotechnology.]

on immature embryos and is limited to one genotype, Tx430 (Liu and Goodwin 2012). Recent advances in seedling-derived leaf-base transformation have the potential to provide genotype flexibility and make this species more accessible for transformation, mutagenesis, and HDR-mediated genome modification (Che et al. 2022; Wang et al. 2023). However, for broader adoption, researchers need a simplified path for access to such patented technologies.

### *Sugarcane*

In sugarcane, each variety must be transformed or edited separately, since introgression by conventional breeding can be difficult (Budeguer et al. 2021). For transformation, one to two months are required to produce tissue for transformation, and another four to five months are needed for production of edited plantlets.

### *Switchgrass*

Most switchgrass protocols start with seed-derived callus (Li and Qu 2011). However, switchgrass does not breed true from seed, so the use of mature-seed-derived callus for transformation is of limited use for functional genomics. A few regenerable genotypes exist that can be used for functional genomics, but transformation and regeneration from mature explants is slow and labor-intensive (Ondzighi-Assoume et al. 2019). *In planta* transformation or more sophisticated editing methods are needed for the future. Increased throughput in terms of numbers of events for screening is needed (Xu et al. 2022).

## Model Plants Relevant to Bioenergy

In addition to the bioenergy crops under investigation by DOE, there are model systems used for transformation to evaluate gene function. These models are smaller in stature, and some are easier to transform. They also have high-quality genome resources, with pan-genomic resources available or under development; mutant germplasm collections; and in-use diversity collections. Ultimately, these models can provide unique opportunities to unravel the complexities of various biochemical and physiological pathways, including C4 photosynthesis, toward developing new crop varieties with improved traits.

### *Arabidopsis*

*Arabidopsis thaliana* is a small annual flowering plant, first chosen as a model because of its small genome size of 115 megabase (Mb) and rapid life cycle (Arabidopsis Genome Initiative 2000). *Arabidopsis* is a key model for flowering plants because of its extensive genomic resources and available analyses of molecular gene functions (arabidopsis.org). It is amenable to floral dip transformation (Clough and Bent 1998) but can also be transformed with other methods (Zhang et al. 2006).

### *Brachypodium distachyon*

*Brachypodium distachyon*, a monocot annual model, possesses a small size, short lifecycle, self-fertility, and a small diploid genome. The inbred line Bd21-3 can be efficiently transformed (Vogel and Hill 2008) with *Agrobacterium*-mediated transformation (Alves et al. 2009). There are 23,000 insertional mutants in wide use by the community (Bragg et al. 2012), demonstrating there has been widespread adoption of *B. distachyon* as a model grass (Hasterok et al. 2022).

### *Panicum hallii*

*Panicum hallii* is a close diploid relative of switchgrass (Lovell et al. 2018), from which it is 8.4 megaannum (Ma) diverged (Lovell et al. 2021). *P. hallii* is a self-compatible, inbred, C4 perennial. Recently, progress has been made transforming multiple genotypes with an efficiency of ~15%. Transformation takes four months from transformation to plants, and there are plant sterility issues to overcome. A fast neutron mutagenized population combined with a new transformation protocol enables rapid testing and complementation of potential gene targets in miscanthus and switchgrass (Kankshita Swaminathan, personal communication).

### *Setaria viridis*

*Setaria viridis* is a panicoid annual evolutionarily related to maize, sorghum, sugarcane, switchgrass, and miscanthus. *S. viridis* is being used as a model plant for monocots due to its short stature, small genome size, rapid life cycle, availability of genome sequences, and repositories of geographically diverse and ecologically distinct accessions (Mamidi et al. 2020). *Agrobacterium*-mediated transformation of callus derived from mature seeds and leaf tissues has been the most reliable and efficient method for recovering stable transgenic plants (Finley et al. 2021; Van Eck 2018).

## Food Crops

Beyond bioenergy crops and model systems, maize and soybean are food crops and are not designated as bioenergy crops by DOE. However, they do contribute to the bioenergy bioeconomy, particularly as sources of current biofuels. These two species have a longer transformation history than the above bioenergy crops, and thus illustrate both potential trajectories and ongoing challenges in transformation.

### *Maize*

Recent advances in seedling-derived leaf-base transformation (Wang et al. 2023) have the potential to make maize more accessible for both random transformation and more controlled outcomes, such as directed mutagenesis, targeted integration via nonhomologous

end-joining (NHEJ), and precise insertions through HDR. The methods need further simplification and dissemination.

#### *Soybean*

Standard transformation methods for soybean are well-established (Parrott and Clemente 2004). However, limiting factors include the costs to generate each transgenic event and for greenhouse space. Moreover, current methods for stable event production are inefficient, labor-intensive, and time consuming. As is the case for many bioenergy crops, soybean shows very little genotype-flexibility.

## Bioenergy Crop Facilities

Given the burgeoning demand for transformation, tissue culture, and genome-modification services, additional capacity in the form of transformation centers will be needed. Only a few transformation centers have been established nationwide, and most are associated with land grant universities. A review of the U.S. plant transformation centers by the NSF Research Coordination Network (RCN) PlantGENE shows that most centers offer services for a handful of widely researched plant species, but only a few offer services for bioenergy crop transformation, with limited available capacity (see Table 2.1, p. 10 and map, p. 11). In addition to specializing in only a few crops, existing facilities also require subsidies to offset rising costs 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. To address these challenges, workshop participants identified ways to enhance plant transformation facilities by creating a coordinated network of DOE-funded plant transformation centers and implementing long-term strategic initiatives.

### *Assessing Current Bioenergy Crop Facilities*

#### *Crop Specialization and Limited Time and Resources*

Over time, plant breeders have developed increasingly sophisticated techniques to conventionally introduce specific traits into breeding populations. However, such breeding programs often rely on availability and access to natural variation that can be time consuming, costly, and labor-intensive to introgress into elite germplasm. Recent developments in genome-modification technologies, plant genetic engineering, and editing can advance the development of new traits that are not easily achievable through more traditional methods, highlighting the importance of plant transformation in multiple areas, from functional genomics to crop improvement.

Because methods can be very species- and genotype-specific, specializing in a broad array of crops is challenging for any one center. Different crops can have widely different growth, transformation, regeneration, and greenhouse requirements. Developing a standard transformation pipeline or onboarding methods based on new technologies requires multiple years before they can be offered as a service. In addition, a dwindling pool of trained researchers, limited physical and infrastructure capacity, and limited funding to purchase new or replace aging equipment have significantly impeded the quality, capacity, and diversity of the services these facilities can offer. These issues also compound the challenge for one facility or laboratory to offer a wider range of crop specialization.

#### *Reliant on Subsidies*

Costs to maintain and operate transformation facilities have risen considerably over the years. Although university or research center–based transformation facilities charge to cover their costs for effort, they still require substantial internal subsidies to sustain operations. As a result, outside users are typically required to pay much higher rates to use these centers, which makes them largely inaccessible to many researchers outside of the institution where they are located. At the same time, there is limited funding for developing and implementing new transformation technologies, thus ensuring that the older, inefficient technologies remain in place. While advances in new technologies, such as morphogenetic genes to enhance genome editing (Che et al. 2022), are promising, access and broader adoption of these technologies have been minimal, in part due to IP restrictions.

### *Enhancing Plant Transformation Facilities*

In the future, a coordinated network of DOE-funded transformation centers, each with its own crop

specialties, may better accommodate transformation needs. 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. Transformation centers would ideally also support ancillary technologies for the research community. A case in point, and as mentioned earlier, many bioenergy crops are cross-pollinated and do not breed true, meaning they must be stored as vegetative propagules. Thus, the long-term storage capabilities of centralized cryopreservation facilities would enable further research in these crops.

Such centers should also have designated field space to facilitate transgenic field trials, given that the isolation and monitoring requirements associated with cross-pollinated crop permits are beyond the capabilities of most academic programs. Furthermore, transformation facilities must be able to assemble vectors, implement quality control measures along the pipeline, negotiate IP, and implement stewardship measures (for more information, see Chapter 5: Intellectual Property, Regulatory Landscape, and Stewardship, p. 18).

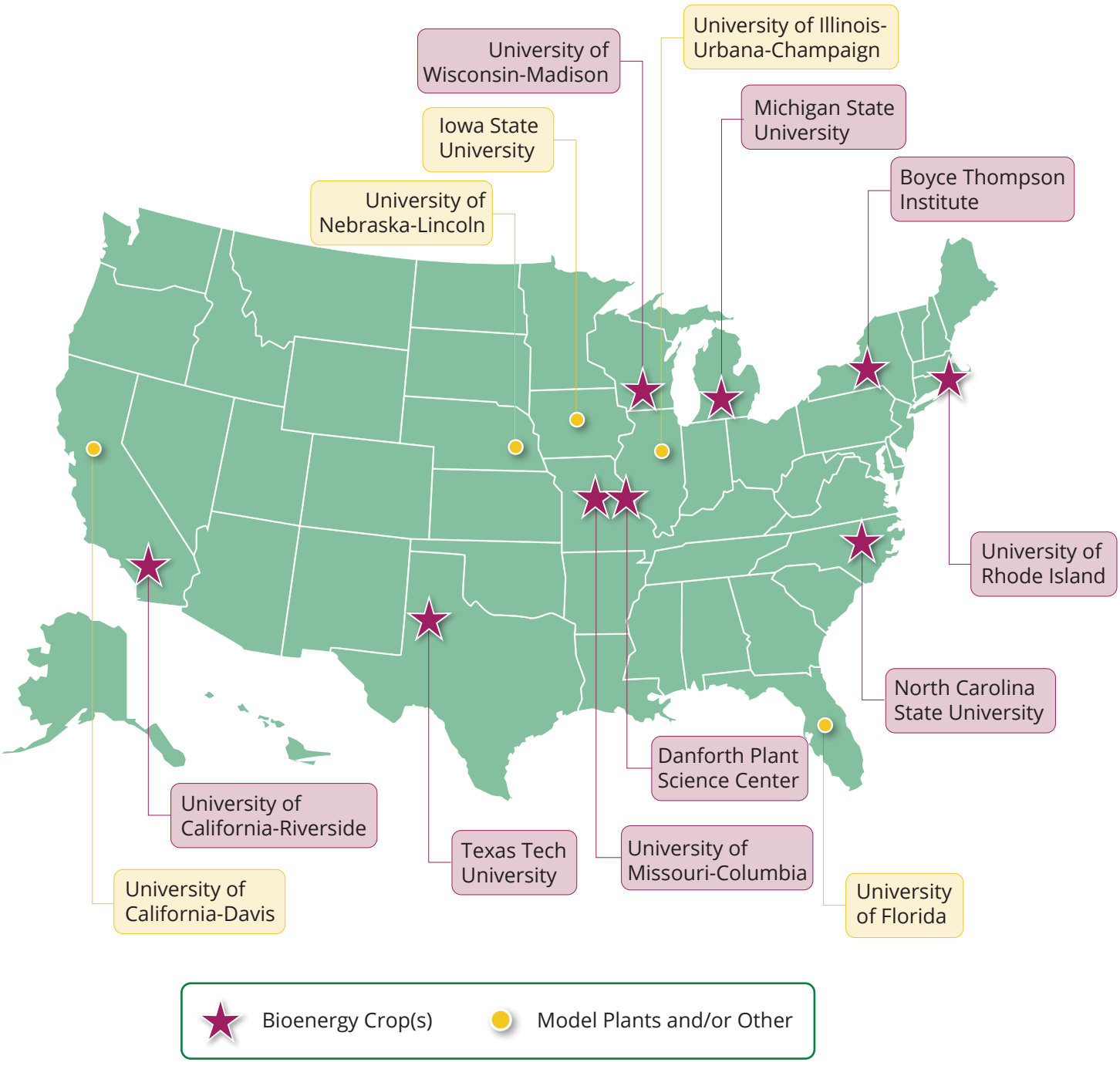
However, transformation centers alone will not solve current transformation limitations. Long-term strategic initiatives will also be needed to support additional research into tissue culture and regeneration biology and the development of new technologies, including the development and incorporation of automation and artificial intelligence. Also needed are training and retention of a skilled workforce with expertise in tissue culture and transformation technologies. Ideally, these long-term strategies would provide standardized policy or guidelines for a standard transformation pipeline, quality control, vector construction, and available strains, among other factors that affect transformation.

## U.S. Transformation Facilities

| Institute/University | Transformation Pipelines | | |
|---|---|---|---|
| | Bioenergy | Model Plants | Other |
| Boyce Thompson Institute, Ithaca, NY | Switchgrass | *Brachypodium*, *Setaria* | Medicago, Potato, Tobacco, Tomato |
| Danforth Plant Science Center, St. Louis, MO | Sorghum | *Setaria* | Maize, Soybean |
| Iowa State University, Ames, IA | — | — | Maize |
| Michigan State University, East Lansing, MI | Switchgrass | — | Apple, Atropa, Blueberry, Canola, Celery, Cherry, Petunia, Rice, Rutabaga, Tobacco, Tomato |
| University of Nebraska-Lincoln, Lincoln, NE | — | — | Soybean, Wheat |
| North Carolina State University, Raleigh, NC | Silvergrass (*Miscanthus*) | *Arabidopsis* | Apple, Cotton, Lettuce, Maize, Melon, Potato, Rose, Soybean, Strawberry, Sweet Potato, Tobacco, Tomato |
| University of California-Davis, Davis, CA | — | — | Medicago (Alfalfa), Canola, Citrus, Grape, Lettuce, Petunia, Rice, Tobacco, Tomato, Wheat |
| University of California-Riverside, Riverside, CA | Poplar | *Arabidopsis* | Lettuce, Canola, Citrus, Medicago, Potato, Rice, Tobacco, Tomato |
| University of Florida, Lake Alfred, FL | — | — | Citrus |
| University of Missouri, Columbia, MO | Sorghum, Switchgrass | *Brachypodium* | Cotton, Maize, Rice, Soybean, Tomato |
| University of Rhode Island, Kingston, RI | Sorghum, Switchgrass | — | Maize, Rice, Tobacco, Turfgrasses, Wheat |
| University of Wisconsin-Madison, Madison, WI | Sorghum | — | Cowpea, Hemp, Maize, Soybean |
| University of Illinois-Urbana-Champaign, Champaign, IL | — | — | Soybean, Tobacco |
| Texas Tech University, Lubbock, TX | Sorghum | — | Cotton, Maize, Rice, Soybean |

**Table 2.1:** List of U.S. transformation facilities that offer transformation services for bioenergy and other crops. Courtesy of PlantGENE (plantgene.atlassian.net/wiki/spaces/PH/overview).

# Transformation Facilities



Chapter 3

# New Methods for Gene Delivery, Transformation, and Regeneration

The impact of CRISPR/Cas on the scale of transformation within bioenergy crops is two-fold: (1) as the methods become easier and more accessible, the volume of CRISPR/Cas work will increase and (2) as CRISPR/Cas-mediated genome modifications become more complex and increasingly incorporate omics approaches (further described in Chapter 4: Leveraging Omics Approaches to Develop Future Transformation Technologies, p. 15), the numbers of T0 plants needed to find the desired modification will increase dramatically. CRISPR/Cas-mediated genome editing can be used for simple mutagenesis or for more complex types of modifications, such as deletions, inversions, small-scale (20–80 base) edits using single-strand oligonucleotides as the template, or longer sequence integrations through either homology-dependent repair (HDR) or nonhomologous end joining (NHEJ).

Different types of CRISPR/Cas-mediated applications have different efficiencies, with mutagenesis being the most efficient, and serves as a baseline for comparisons to modifications made with *Agrobacterium* delivery of Cas, gRNA(s), and *Wus2/Bbm* morphogenic genes. Svitashev et al. (2015) reported that multibase editing using single-stranded oligonucleotides was roughly 10-fold less efficient than integrating larger sequences through HDR (0.02–0.04% vs. 0.7–4.0%, respectively). In a separate study, Gao et al. (2020b) demonstrated that deletion of an endogenous maize gene (~ 6 kb) was observed at an average frequency of 10% (across 10 different maize inbreds). Finally, in Barone et al. (2020), single-site mutagenesis frequencies ranging between 75–95% were observed along with targeted integration through HDR at either 2.7% or 4.7%. These data can predict the number of transgenic T0 plants a laboratory would need to produce to recover a given number of plants with the desired outcome.

To produce three T0 plants containing the desired outcome for either mutagenesis, deletions, HDR-mediated integration, or oligonucleotide-mediated editing, a laboratory would need to produce a total of approximately 3–4, 30, 100, or 1000 T0 plants, respectively, and then screen the plants using polymerase chain reaction to find the desired plants. These are extrapolations; currently, almost all CRISPR/Cas-mediated editing is focused on mutagenesis. However, future needs are predicated on performing increasingly complex cisgenic and intragenic modifications, which must be anticipated when assessing future needs in bioenergy crop transformation. Ultimately, HDR alone may not be the method of choice for the insertion of large DNA segments into specific genomic locations, and new alternatives have been proposed, such as CRISPR-associated transposases (CAST; Liu et al. preprint), PrimeRoot (Sun et al. 2024), programmable addition via site-specific targeting elements (PASTE; Yarnall et al. 2023), and most recently, CRISPR/Cas-guided exonucleases that enhance HDR (Schreiber et al. 2024). However, all of these alternatives need further development. The following section outlines new methods that could make transformation more efficient.

## New Methods to Increase Transformation Efficiency

To meet anticipated needs, bioenergy crop transformation will require significant improvements in DNA delivery, the speed and efficiency of recovering stable transgenic regenerable tissues or cell lines, and high-throughput detailed molecular characterization. Tissue culture-free methods also show promise for improving the efficiency of bioenergy crop transformation.

## Gene Delivery and Regeneration Methods

The biology and genetic mechanisms underlying plant regeneration are not well understood. Short- and long-term strategies to increase editing and transformation efficiencies should help provide the throughput needed to gain a deeper understanding of regeneration, including (but not limited to) somatic embryogenesis, DNA repair, and 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. For example, the discovery of additional growth regulator genes could improve regeneration (Gordon-Kamm et al. 2019; Nalapalli et al. 2021). DNA or endonuclease delivery could be facilitated by: (1) modifying crops to be amenable to floral dip (Liu et al. 2012); (2) using viral delivery of sgRNAs (Ellison et al. 2020; Gong et al. 2021) and Cas nucleases (Gong et al. 2024); (3) perfecting nanoparticles for reagent delivery (Demirer et al. 2019); and (4) developing improved *Agrobacterium* strains, especially those that avoid incompatibility reactions with the plant (De Saeger et al. 2021). The level of achievable modifications would benefit from artificial chromosome technology and tunable or synthetic promoters. Modification of organellar genomes may be particularly useful to enable particular traits and phenotypes (Maliga and Bock 2011).

Type, quality, and methods used to prepare explants (e.g., donor materials) are critical to successful transformation. Substantial improvement can also be made when using explants that are compatible with bioenergy crops. Here, the use of *Bbm/Wus* may provide the key toward future bioenergy crop transformation and regeneration improvements that fall within the grass family. Recent work has demonstrated that using these morphogenic genes permits efficient transformation (and genome editing) using *Agrobacterium* to introduce T-DNA into fragmented leaf tissue of maize and sorghum. This method may be applicable to many crops within the Poaceae (Wang et al. 2023).

Similarly, the newly developed *Lec2/Bbm* method has been demonstrated to result in hormone-free rapid formation of somatic embryos and regeneration across a broad range of plant species and genotypes, such as recalcitrant cotton, cucumber, California poppy, and tomato (Cho et al. 2023). Other morphogenic regulatory gene combinations also have shown promise for improving transformation. Examples include use of *GRF4/GIF1* (Debernardi et al. 2020) or *GRF* genes alone (Kong et al. 2020) in both monocot and dicot species, or use of *Wus/IPT* in tobacco (Maher et al. 2020). Further research on morphogenic genes is anticipated to expand genotype range, increase transformation efficiencies, and reduce cycle times for transformation and editing.

Optimal use of morphogenic genes is not technically difficult, but an efficient and successful outcome depends on attending to details within the protocol and understanding why these details matter. The importance of these details, which often goes unnoticed, impacts technology transfer and extending morphogenesis technologies to new plant species and genotypes. As a result, transferring this technology to new laboratories has often been difficult and slow (William Gordon-Kamm, personal observation). Accordingly, establishing optimized techniques across a network of laboratories and facilities will require a combination of hands-on workshops and virtual training. In addition, time, resources, and continued interaction between trainers and trainees are needed given the learning curve.

Extending such methods to new crops or genotypes is challenging due to the need to adjust multiple variables. These adjustments make difficult combinatorial testing requisite. Looking to the future, AI combined with automation may provide new solutions to such complexity. Further, combining tools such as nanotechnologies and AI-driven hyperspectral imaging and decision-making may further enhance progress in these historically intractable areas. Rationally combining these tools by employing Design of Experiment approaches (Niedz and Evens 2016), along with automation and new imaging platforms, may accelerate progress toward the goal of generating large numbers of transgenic events in a short period of time with minimal resources.

Ultimately, tissue culture and regeneration remain extremely labor-intensive technologies that require very particular training. While many aspects of

genomics have become automated, the same is not yet true of tissue culture, in part, because of the need to be able to recognize and transfer very specific types of tissue. As these labor requirements are one of the largest constraints to more efficient tissue culture and regeneration systems, advances in robotics, coupled with AI-enabled machine vision, could revolutionize tissue culture. Combinations of these technologies would rapidly advance the field of transformation, for example, by incorporation of physical or *Agrobacterium*-mediated delivery of viral components that then replicate and move editing components into neighboring or even distant cells.

## High-Throughput Molecular Screening Methods

Such improvements in rapid high-efficiency transformation need to be complemented with a new generation of high-throughput molecular screening tools for early identification of quality events in the transformation process. These advanced screening tools would also permit all other events to be discarded early in the process, before spending additional resources to culture and grow events that will ultimately be discarded. Such identification could be accomplished through AI-mediated process control coupled with robotics for sampling, extraction, and nucleic acid analysis. For rapid quality control and sequencing to validate edits and assess potential genome rearrangements, DOE currently has the necessary sequencing capacity and could deploy a centralized service applying short- and long-read sequencing to edited material. However, this application may require additional investment for robust service and turnaround times.

## Tissue Culture-Free Transformation Methods

While the use of morphogenic genes can make transformation more efficient and rapid, these approaches still involve generation of large, complex vectors and labor-intensive tissue culture methods to produce transgenic or edited plants. Tissue culture-free methods could greatly simplify the process of generating transgenic plants, which would dramatically reduce overall costs, training, and infrastructure needs. Maher et al. (2020) successfully used the *Wus/IPT* gene combination in tobacco to demonstrate direct transgene-mediated axillary shoot formation to produce *de novo* edited plants. However, this method still needs optimization to produce higher efficiencies and to be extended to other plant species.

Another exciting example is the cut-dip method recently demonstrated in sweet potato. In this method, the plant's roots were removed and the rootless plant was dipped in *Agrobacterium* and then thrust back into the soil. Root proliferation through suckering and new shoot formation from the suckers completed the process of transgenic plant recovery (Cao et al. 2022). Additional work would be needed to remove the rhizogenes oncogenes, the *rol* genes associated with hairy roots. While not immediately transferable to species that do not propagate through root proliferation, these methods may be worth exploring for bioenergy crops such as poplar. For other mainstream crops, new breakthroughs await discovery.

Chapter 4

# Leveraging Omics Approaches to Develop Future Transformation Technologies

Since the first *Arabidopsis* genome sequence was produced 24 years ago, plant genomics has come a long way. Thousands of reference genome sequences have been produced across species of interest, and the plant genomics world has adopted techniques for functional assays from both model plant species and mammalian genomics research. Despite these advances, the much needed centralized and high-capacity plant transformation facilities cannot be created without a major research effort to understand the plant biology involved. Modifying or improving plant function through transformation requires two important pieces of information: (1) a deep understanding of the relevant biological pathways and their functions and (2) a detailed, high-quality annotated reference genome to enable targeting or changes to these pathways.

Increased knowledge of plant functional elements can be incorporated into designs for bioenergy crop editing and pathway modification. Several applications require high-quality reference genome sequences for lines used in transformation and editing pipelines. Examples of such applications include advancing structural annotation to further improve selection of editing targets in complex genes with multiple family members and alternative splice variants. High-quality reference pan-genomes can identify safe harbors and help select genomic locations for site-directed integration and landing pad installation. However, current limitations exist that impede such improvements to transformation. This chapter identifies these limitations and discusses new genomics tools and technologies that can help accelerate improvements to bioenergy crop transformation.

## Current Limitations to Transformation Improvements

Improvements to transformation in most bioenergy crops are considerably hindered by limited data on full genome sequences, gene function, promoters, and regulatory elements—for genes in general and for polyploid genes in particular. Evolutionary history of the species has shaped polyploid genes, which have undergone sub-functionalization, neo-functionalization, and pseudogenization. As a result, polyploid gene functions will not always be directly inferable from functional studies in a model plant system.

Bioenergy crop transformation improvements are also inhibited by a lack of understanding about how promoters, enhancers, and transcription factor binding sites, such as cis-regulatory elements (CREs), impact the position effects of transgene insertions. CREs need to be identified to construct pathways that link into existing transcription factors. Also needed are sequences that show insulator-like function and can dampen ectopic interactions between transgenes in a construct (Laspisa et al. 2023). With this expanded knowledge, tissue-specific promoters and other regulatory sequences can be used to express pathways in specific tissues, under specific conditions, and ultimately take engineering closer to practical application in these species.

## New Tools and Technologies

New genomic tools will continue to accelerate discovery of new genes and epigenetic factors that can be manipulated to improve the speed and efficiency of transformation in a broad range of plant species,

including bioenergy crops. These approaches can enhance functional insights, resolve long-standing questions, and increase knowledge of bioenergy crop biology. Furthermore, machine learning (ML), artificial intelligence (AI), and robotics can be leveraged to assist in plant transformation advancements.

## Enhance Functional Insights

To improve understanding of gene function, promoters, and regulatory elements in bioenergy crops, new genomic tools now enable the generation of extremely accurate genome sequences that precisely define polyploid bioenergy crop gene variants resulting from divergence over time. Production of highly contiguous haplotype-resolved genome references (e.g., in outbred switchgrass) is now possible with the availability of high-quality reference genomes. These new genome references allow longer single molecule sequencing reads to completely resolve the homoeologous gene copies represented by two parental subgenomes, which can be phased into two haplotypes. The result is a reference genome in switchgrass with four copies of each gene, on average (Juenger and Schmutz, unpublished). These new references, such as those for poplar (Zhou et al. 2023), allow precise targeting of gene edits to one subgenome or across haplotypes to capture gene family variations (Bewg et al. 2022).

Gene function and regulatory elements in bioenergy crops can be further understood through multiple new genomics approaches. In addition to standard tools for expression such as RNA-sequencing, single-cell RNA sequencing (scRNA-seq; Tang et al. 2009) can profile expression differences and allow interrogation of individual cell types in a plant sample. Assay for Transposase-Accessible Chromatin with sequencing (ATAC-seq; Buenrostro et al. 2013) can be used to determine expression potential for all the genes in the genome for a specific tissue or condition. This resolution can be further improved with single-cell sequencing assay for Transposase-Accessible Chromatin (scATAC-seq; Buenrostro et al. 2015), which identifies these accessible genes on a per cell type basis. To identify transcription factor binding sites and improve gene interaction predictions, DNA affinity purification sequencing (DAP-seq) can be applied to any transcription factor or genotype combination (Bartlett et al. 2017).

## Resolve Long-Standing Questions

The application of genomic tools can greatly inform transformation abilities by resolving long-standing plant genetics questions, such as recalcitrance to transformation, self-incompatibility, and reproduction. Genotype-specific transformation is a major issue. In bioenergy crops, the common sorghum transformation genotype is TX430, in poplar it is INRA 717-1B4, and in switchgrass it is a synthetic cultivar called 'Alamo.' These differences in efficiencies, including transformation recalcitrance of other genotypes, are driven by unknown, underlying genetic or epigenetic variation. Understanding this underlying variation would enable more widespread adoption of plant engineering. It would also improve applicability. Typically, the easiest lines to transform are not elite lines, which dictate that any edits or transgenes then need to be crossed into production lines. In the case of bioenergy crops, the resultant timelines can be quite long or not even possible. For example, triploid grasses are largely sterile, which obviates crossing as a strategy to introduce new variants.

Additionally, self-incompatibility prevents self-fertilization of plants and accordingly promotes outcrossing and reduces inbreeding. Despite ongoing efforts, major bioenergy grasses including switchgrass and *Miscanthus*, as well as poplar, are all self-incompatible. Multiple molecular mechanisms of self-incompatibility have been identified across plants (Muñoz-Sanz et al. 2020), but the types of incompatibility operating in bioenergy crops have not been characterized. If it is possible to identify the self-incompatibility mechanisms for bioenergy crops, we can then engineer these systems to produce editable, self-fertilizing lines, which would in turn enable hybrid breeding programs to maximize production and feedstock uniformity. Finally, understanding key reproduction traits for bioenergy crops could enable deployment strategies that allow for controlled crossing while limiting gene flow and for development of male sterile lines for breeding programs (Daniell 2002).

## Increase Knowledge of Bioenergy Crop Biology

Use of modern genomic tools can vastly increase understanding of bioenergy crop biology; improve

target selection and transformation experiment design; and yield faster, more insightful data from the long transformation timeline through better prediction and design. These approaches can also be used to directly study plant transformation. Recent substantive advances have come from applying genomic techniques to identify key developmental regulators throughout the tissue culture process. These are then applied as external factors to improve transformability of difficult genotypes and increase overall transformation efficiency (Lowe et al. 2016).

Model plants have undergone extensive genomics work in transcriptomics, proteomics, and metabolomics. While a fundamental understanding of genetic regulation of flowering and pollination exists, transfer of this knowledge into bioenergy crops has been limited. Knowledge transfer to bioenergy species is particularly complicated by limitations in gene functional knowledge, polyploidy, and difficulties determining gene fuction in a heterozygous background.

## *Leverage Machine Learning, AI-Assisted Plant Transformation, and Robotics*

Much higher transformation frequencies will be required to generate hundreds of events per transformation (see Chapter 3: New Methods for Gene Delivery, Transformation, and Regeneration, p. 12). High-throughput molecular screening tools for early and rapid quality event screening are essential, as maintaining unscreened plants in the greenhouse that will later be discarded becomes cost-prohibitive. While the number of events needed for research and development are high; those for event commercialization need to be even greater.

Screening such high plantlet numbers to identify the one to few optimally edited plants requires a level of centralized and integrated mechanized tissue culture and molecular screening that is currently unavailable. Examples of such technology include: (1) combining hyperspectral imaging in tissue culture, (2) merging robotics with AI or ML to identify individual healthy plantlets, (3) mechanizing the sampling process, and (4) running automated polymerase chain reaction or next-generation sequencing as molecular screens. Leveraging ML, AI, and robotics to generate such advanced tools would contribute toward the final objective: identifying quality transgenic, or nontransgenic but successfully edited, individual plantlets for transfer to soil and the greenhouse.

ML, AI, and robotics could also help increase the efficiency of the transformation pipeline. As a whole, the transformation pipeline moves from the transformation laboratory, into molecular analysis, and finally into the greenhouse (see Fig. ES.1, p. vi). This pipeline often relies on separate databases, which must be integrated to ensure event tracking through the system, data continuity, and identity preservation for each newly created T0 event. In an ideal scenario, data integration would happen through a centralized, user-friendly platform that would allow facile data retrieval and report generation. However, additional resources are needed to create such a system. While such a scenario is currently beyond the capabilities of individual academic laboratories, it could be feasible for a centrally funded research facility focused on developing such pipelines.

Chapter 5

# Intellectual Property, Regulatory Landscape, and Stewardship

## Intellectual Property

All transformation facilities—and, for that matter, all researchers—need to know the issues associated with intellectual property (IP) and how it impacts their ability to conduct research. IP can apply to (1) the genes and vectors, (2) the methods and processes used, and (3) the plant variety being transformed or edited. Contrary to commonly held beliefs, there is no research exemption, so all pertinent technologies need to be licensed. Sometimes, the best or most efficient protocol will not be available because of its associated IP. In addition, for traits destined for commercialization, the transformation facility or researcher could incur triple damages if they do not first obtain freedom to operate by licensing the necessary technologies.

This area is complex and nuanced, dependent on the specific technology, the IP assignee, and the entity seeking a license. These complexities make general guidance difficult. If specific technologies, such as use of morphogenic genes, are critical to the advancement of transformation or gene editing in bioenergy crops, establishing a network of researchers could be useful to not only increase group knowledge of the issues but also develop group strategies to expand technological access for the entire community.

Open protocols and public vectors are a necessity in public user facilities to increase the ability to train and disseminate protocols to the wider transformation community. A previous attempt, the Public Intellectual Property Resource for Agriculture (PIPRA), serves as a model through which this objective can be accomplished (Chi-Ham et al. 2012). Administering a similar initiative could fall under the auspices of a bioenergy crop transformation center.

## Regulatory Considerations

All plant work with recombinant DNA (rDNA) is regulated, though some agencies exempt some modifications. The first priority of regulation is to ensure that organisms containing rDNA are not inadvertently released. Therefore, all experimental protocols must be reviewed by an Institutional Biosafety Committee (IBC) to confirm compliance with National Institutes of Health (NIH) guidelines on rDNA. All institutions that receive any NIH funding—such as universities hosting plant transformation facilities—are required to have an IBC. In fact, having an IBC has also become standard practice in both private and public institutions, even if they do not receive NIH funding.

In the U.S., three main agencies regulate transgenic plants upon leaving the transformation facility: the U.S. Department of Agriculture (USDA), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). Transformation facilities are likely to first be impacted by USDA regulations, as they affect materials moving out of a confined space in a laboratory or greenhouse and into a field or across state lines. The USDA's position is that all materials with rDNA are regulated unless determined otherwise. In limited cases, a developer can self-determine if their product falls within one of the exempt categories defined by USDA's Animal and Plant Health Inspection Service (USDA APHIS 2020). Examples include situations when only one DNA cut was made with an endonuclease, when a cisgene has been used, when the gene (or an orthologous gene with the same mechanism of action) has been previously deregulated, or when no foreign DNA remains in the genome. As of this writing, the USDA is considering increasing the number of allowable simultaneous edits of a genome from one to four.

Facilities requiring clarification about a product's regulatory status can request a status confirmation from the USDA. If the product is regulated, the transformation facility can follow two options before shipping the transgenic or edited products: (1) it can request a shipping permit, or (2) it can request a regulatory status review to determine if the product is exempt from USDA regulation (USDA APHIS 2024).

The USDA will base its regulatory decision on an edited or transgenic plant's potential to increase plant pests. Several bioenergy crops have conspecific relatives in the U.S., so gene flow that results in hybrids with altered phenology is at least theoretically possible. Creating sterile bioenergy crops would help ease the USDA's plant pest concerns. The development of conditional or reversible sterility systems would be more beneficial because these systems would still allow for seed production and downstream breeding of improved cultivars. In all cases, whether regulated or exempt, shipping must adhere to the USDA's shipping standards for recombinant organisms.

Other regulations come into effect once the edited or transgenic plant has left the transformation facility. Bioenergy crops that can also be used as forages for livestock may go through an FDA consultation. Additionally, barring narrow exceptions, the EPA will regulate any transgene or edit that results in resistance to a disease or pest. Although EPA regulations do not start until more than 10 acres are planted, the agency still needs to be notified of any field trials. Nonexempt products need to be registered as pesticides. Moreover, the EPA currently considers changes to plant growth and development from editing or transgenesis to be a plant growth regulator, which qualifies as a category of pesticide. Thus, many of the growth alterations being considered for bioenergy crops could also be regulated by the EPA and require registration as pesticides.

## Stewardship

Based on past incidents, the unintentional release of noncommercialized edits or transgenes—whether regulated or not—generates adverse media coverage and turns public opinion against biotechnology. International commerce can also be threatened. Events not regulated in the United States will almost inevitably be regulated somewhere, so the presence of unapproved events in food exports can and has resulted in economic losses and substantial liability.

It can be surprisingly easy for transgenic or edited pollen, seeds, or other reproductive propagules to escape confinement, as there are several possible escape points for transgenes and edits during seed handling, storage, and greenhouse propagation. For example, seed can be mixed or mislabeled during handling and storage, and a stray insect can carry pollen out of the greenhouse. Hence, every establishment involved in editing should identify the possible escape points and have a set of standard operating procedures in place to minimize the possibility of unintentional release of edited or transgenic pollen, seeds, or other reproductive propagules. Establishments should also ensure all personnel are properly trained and aware of the need for stewardship. As mislabeled materials can result in the inadvertent release of edited or transgenic plants, proper track and trace documentation such as identification of cell lines, vectors, and plants throughout the production process is crucial.

Once these plants leave the laboratory and have entered commercial production, the need for reproduction control technology (see Chapter 3: Leveraging Omics Approaches to Develop Future Transformation, p. 12) will be key for stewardship efforts. Ideally, for bioenergy crop production, scientists would be able to engineer appropriate biocontrol methods into feedstocks prior to deployment. Such reproductive controls would ease concerns over potential outcrossing into remaining native populations of these plants.

Ultimately, both regulations and the laws that govern IP are complex and nuanced, requiring substantial expertise to understand. As such, training resources and opportunities must be available. Every user or organization could inadvertently provide a potential escape point, so everyone needs to be well-versed in stewardship and have access to experts or consultants who can provide guidance related to IP laws for transformation technologies and regulatory policies for handling of transgenic materials.

Chapter 6

# Developing an Inclusive Community and Talent Pool

Meeting the growing demands of the plant transformation community will require building and training a highly skilled talent pool. Not only will this workforce need to be competent in a diverse range of tasks and challenges, it also must leverage the talent of students and researchers from across all of society, including groups historically underrepresented in science, engineering, and technology. This chapter describes current challenges in plant transformation training and identifies opportunities to develop an inclusive and skilled community and workforce now and in the future.

Transformation laboratories and facilities require human labor with diverse levels of training. Although tissue transfer work does not require advanced background knowledge, it does require staff with meticulous attention to detail and skilled in the art of plant tissue culture and transformation. Also needed are researchers with enough knowledge, education, and skill to advance the science of plant transformation and regeneration, troubleshoot existing protocols, and design entirely new ones based on emerging knowledge. With the advent of robotics and artificial intelligence (AI), these scientists should also be trained to work with engineers to develop applications for plant tissue culture and genetic engineering. In addition, scientists with advanced degrees in molecular genetics and cell biology are needed to discover the fundamentals of transformation, recalcitrance, and regeneration.

The inability to find and maintain trained personnel for these different needs is a limitation affecting all plant transformation laboratories and facilities. Compared to the biomedical field, most students are not aware of plant science career opportunities, and even if they are, the necessary training infrastructure and its funding are very limited. A concerted effort is needed to provide role models, develop the needed curricula, and dedicate funding for training opportunities.

## Current Training Challenges

Workshop attendees agreed that the plant transformation field lacks an adequate training pipeline despite considerable demand for staff expertise in basic research, translational research, and applied aspects of transformation and gene editing. Moreover, without concerted outreach to historically underrepresented groups in plant transformation science, the field is unlikely to have enough skilled labor in the future.

### *University Practices and Approaches*

Too few universities are invested in plant biotechnology, even among the land-grant universities. One challenge is that training in plant transformation and editing is often considered to be a technical problem versus a biological research problem. Faculty may use transgenic and editing methods and materials, but their funded research typically is directed toward basic and applied hypothesis-driven biological challenges, and they often view transformation as a tool rather than as a biological research problem in its own right. Moreover, there is very limited opportunity for obtaining tenure-track positions that center around the technical aspects of plant transformation technologies and the training for them. Thus, research and training relevant to advancing the basic science underlying plant transformation are often not a primary focus.

For those universities and core facilities that do train students and staff in relevant technologies, turnover can be high. Anecdotal evidence suggests this is related to these institutions' inability to compete with industry pay scales. Furthermore, training takes time and can be constrained by university policies, such as salary freezes due to budgetary issues. Universities also face pressure to train a disproportionate number of doctoral students compared to technicians.

Another challenge is that federal grants may emphasize training for graduate students and postdoctoral staff, but transformation facilities are highly unlikely to hire postdoctoral researchers to do routine work. Also, the COVID-19 pandemic negatively affected the already-rudimentary training pipeline as virtual courses reduced opportunities for hands-on activities crucial for plant transformation training.

### *Diversifying the Plant Transformation Workforce*

Inclusivity includes race, ethnicity, ability, gender, socioeconomic status, and more. The plant transformation community appears diverse in terms of international participants, women, and minorities. However, the training of underrepresented minorities in agricultural or biological sciences lags behind other nonminority groups. In these fields, minorities earn fewer than 25%, 20%, and 15% of bachelor's, master's, and doctoral degrees, respectively (NSF 2022). At the community college level, they earn about 44% of associate degrees in biological sciences and only 12% in agricultural sciences (NSF 2022).

Workshop participants noted that the culture of individual facilities can impact staff diversity: a more diverse staff attracts more diversity (Dauth et al. 2023; Dixon-Fyle et al. 2020). However, social acceptance may not be the same as inclusivity (Garrote et al. 2020). Also, some aspects of plant transformation research—such as the need for confidentiality related to intellectual property (IP), nondisclosure agreements, and competition for limited funding—may also impact inclusivity in the plant transformation community. Workshop participants themselves represented an inclusive and diverse group and can serve as a model to increase these factors.

## Training Needs and Opportunities

### *Expanding Programs Across all Educational and Career Levels*

Training and career advancement opportunities are needed for technical staff and students at all levels, including entry-level workers, undergraduates at universities and community colleges, graduate students, and postdoctoral researchers with advanced degrees. Moreover, technical positions in transformation facilities need to be salaried at a level commensurate with industry to entice scientists to choose these positions as long-lasting careers.

One strategy to develop the needed talent pool includes improving undergraduate education by offering bachelor's degrees in biotechnology and biomanufacturing. Paid internships at companies and transformation facilities are another possibility. An additional approach could involve an activity like the T32 training program funded by the National Institutes of Health in which pre-doctoral fellows complete paid internships. Such a program could be expanded to undergraduates at universities and community colleges and offer participants the additional benefits of exposure to nonacademic research and new technologies, introduction to IP, networking, and development of a strong curriculum vitae.

Master's degrees in plant biotechnology can also be an attractive route to develop personnel for plant transformation positions. Master's programs that combine didactic and instructive education with experiential learning could serve as excellent sources of trained personnel. Graduates from these programs also would have a sense of the requirements for continuing their education in PhD programs. Career orientations beyond academia (e.g., in industry) could be built into these programs as well.

Paid apprenticeships are an integral part of many technical positions and can be an effective strategy to build the diverse talent pool required to advance plant transformation research and application. Compared to internships, which typically last weeks, apprenticeships last longer (months to years), are more organized, and have clearer goals. As a result, apprentices gain a comprehensive knowledge of systems and skills. Individuals with all levels of education can be trained to do specific and repetitive procedures with detailed instruction. Experienced mentors, including those without a college degree, can provide the requisite training. Expectations include a permanent job for the apprentice upon successful completion of training.

## Increasing Outreach to K–12 Students

Building a pipeline of skilled workers also will require a concerted effort by stakeholders to reach and capture the imagination of younger students. Introducing plant sciences as an interesting field and career option must start and continue throughout grades K–12. University and community college professors, professional societies, and corporations focused on plant biotechnology and agriculture all need to enhance outreach to their K–12 communities. Possible activities include tours that include demonstrations and small take-home experiments; assistance with science fairs and clubs; and other activities, such as "Rent a Professor" visits and experiment demonstrations at schools. Peer mentoring activities between college students and K–12 students are also valuable (Ohlson et al. 2020; Ritter et al. 2009).

## Developing New Curricula and Educational Strategies

Universities and community colleges should develop collaborations that include courses in the basic aspects of transformation, plant tissue culture, and genetic engineering (see Fig. 6.1, this page). In addition, more direct connections need to be developed between educational institutions that have plant science departments and industry, including both small start-ups and major corporations.

Both universities and community colleges are searching for ways to increase course enrollments. One approach to achieve this while strengthening the plant transformation workforce is the development of micro-courses (or micro-credentials) on relevant topics such as tissue culture, transformation, robotics, and regulatory compliance. Institutions with teaching equipment not fully used during summer (e.g., laminar flow hoods and biosafety cabinets) could offer training utilizing this equipment. Such micro-courses would be short, intensive experiences that would include the practical skills required for repetitive transformation and editing. These courses could also be quickly modified as technology changes. For example, as robotics, AI, and engineering technologies become more established, colleges will need to be nimbler in adjusting their transformation-related course offerings to incorporate these fields. These courses would also be attractive to otherwise noncredentialed workers.

**Fig. 6.1. Training the Next-Generation Workforce.** At Elizabeth City State University (ECSU), molecular biology students taught by Dr. Margaret Young perform a plant transformation experiment. From right are Nah'Turie Ward, Kayla Lee, Robert Chase, and Kimberley Toner. [Courtesy Margaret Young, ECSU]

Community colleges are already leading the way in this new educational model. These institutions are particularly effective at tapping into groups underrepresented in science, including rural-area and first-generation college students as well as various ethnic and immigrant groups. Community colleges also could offer retraining programs once industries leave an area. One such example is Alamance Community College in North Carolina, an institution that re-tooled itself after the local textile industry exited. It developed a biotechnology program that now offers trained personnel (including in plant biotechnology) for the Research Triangle Park, a cooperation between North Carolina universities and industry that represents the largest such park in the United States.

Other potential solutions to the talent pool challenge are newer educational strategies called course-based undergraduate research experiences (CUREs), which have been recognized to increase students' science competencies (Linn et al. 2015). Plant transformation CURES could be integrated into the curricula of universities and community colleges. These teaching strategies, which require dedicated funding, could also enhance the productivity of faculty at smaller and more diverse institutions (Staub et al. 2016). For example, CUREs can be used to generate scientific and educational data leading to journal articles (Shortlidge et al. 2016).

## *Funding Training Opportunities*

Funding the training of future technicians and scientists in plant transformation and editing will require the involvement of all entities, including universities and community colleges; transformation facilities, industry, and federal agencies such as DOE, the National Science Foundation (NSF), and the U.S. Department of Agriculture (USDA). Federal training grant opportunities could be designed to further support and enhance activities, such as apprenticeships, internships, faculty programs, and workshops. Tapping into existing programs, such as NSF's Research Experience for Undergraduates and USDA's Research and Extension Experiences for Undergraduates, could also promote cross-training in plant biotechnology.

Finally, another opportunity involves embedding small start-up company activities into universities to increase collaboration. Also, many companies with large agricultural footprints (i.e., "Big Ag") already have connections with land-grant universities. Such relationships need to fully embrace all the strategies herein.

# Chapter 7
# Summary

To overcome current limitations and increase bioenergy crop transformation efficiency and capacity, the public plant genomics field must work toward a community-based solution. Such a solution requires investing in major genomic analysis projects of bioenergy crop transformation and regeneration to effectively create a science focus area that would allow deep understanding and optimal application of transformation and associated technologies. Toward this end, workshop participants envisioned a centralized DOE transformation effort that would provide both large-scale transformation services and coordinated research to understand the basic biology of transformation, regeneration, and recalcitrance. These developments would take the field of plant transformation to the next level.

Workshop participants also identified the need for expanded training programs in plant transformation and editing. Most individual academic laboratories have students interested in studying or improving a crop of interest, rather than on becoming experts in transformation and editing techniques *per se*. At the same time, academic researchers desperately need help in terms of accessibility to technology, financial feasibility, and throughput. This chapter outlines these and other plant transformation needs and opportunities that emerged from the workshop.

## Plant Transformation Needs and Opportunities to Create a Community-Based Solution

Five central themes in plant transformation were identified for their integral roles in creating a community-based solution. They are: (1) a centrally funded DOE research facility, (2) a coordinated network of DOE-funded plant transformation facilities, (3) a diverse workforce in plant transformation, (4) competitive funding opportunities for the research community, and (5) potential partnerships with other federal agencies. Existing transformation facilities will need to be networked and coordinated to maximize their effectiveness while resources 1–5 are being developed.

***1. 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. This facility would also provide openly available research protocols and methodologies.***

A centrally funded DOE research facility would achieve the following goals:

- Determination of the molecular mechanisms underlying plant transformation recalcitrance and regeneration.
- Identification of safe harbor locations (i.e., genome locations where the transgenic modification does not affect nearby genes or regulatory elements) and development of community accessible lines with standardized insertion sites in every relevant bioenergy crop species.
- Design and development of robotics platforms and AI methods for the transformation pipeline and automated phenotyping.
- Solutions for additional plant transformation challenges and support of opportunities 2–5 listed below.

***2. 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.***

These service laboratories would be user facilities located at national laboratories, universities, and academic research centers that provide established transformation services to the academic community.

Academic researchers could access these services either through:

- Competitive grant applications that are modeled after other DOE user facility programs, or
- Federal funding to perform bioenergy research from DOE or from any other U.S. federal agency upon installation of memorandums of understanding.

### *3. Funding and training to develop a diverse workforce in plant transformation coupled with opportunities to attract and retain these skilled researchers.*

Technical positions in these laboratories would be staffed and salaried at an appropriate level that is commensurate with industry, such that scientists would choose these positions as long-lasting careers. Importantly, these laboratories could also help develop the requisite transformation workforce by offering apprenticeships, internships, co-ops, graduate research projects, and community workshops. Indeed, both the DOE research facility and the transformation service laboratories could offer a structured training pipeline that extended from internships and micro-courses to full apprenticeships leading to permanent employment. Ultimately, this scenario could substantially contribute to filling the need for skilled personnel.

### *4. New DOE competitive funding opportunities for the research community to perform basic research on transformation and regeneration biology and methodology should be provided to improve the scale and effectiveness of the above approaches. Such funding could also support training initiatives to expand the transformation workforce.*

### *5. Establishing partnerships with other federal agencies to increase the scope and magnitude of research in bioenergy crop transformation and to assist in workforce development.*

Potential partnerships could include:

- USDA for transformation laboratory sites
- NSF and other federal agencies for funding university basic research in transformation and regeneration
- NSF and other federal agencies for workforce development
- Universities for workforce development and plant transformation innovation.

Ultimately, the development of these five resources coupled with the concurrent networking and coordination of existing transformation facilities will address current limitations and increase efficiency and capacity of bioenergy crop transformation.

The lack of a cohesive, community-wide action plan has left plant transformation capabilities at a crisis point in the U.S. With its history of brick-and-mortar facilities and academic research partnerships, DOE is ideally suited among federal agencies to address this crisis. The advancement of plant transformation technology and capacity is essential to driving innovation and improving the nation's energy security. Future DOE investments in basic and applied transformation research that are comparable to investments in other major DOE-supported disciplines will not only benefit agriculture but also ensure U.S. competitiveness in the emerging bioeconomy.

# Appendix A
# Workshop Agenda

## Overcoming Barriers in Plant Transformation
### A Focus on Bioenergy Crops
### Virtual Workshop
**September 18–20, 2023**

***All times Eastern***

## Monday, September 18

| | |
|---|---|
| 11:00–11:15 a.m. | **Introduction and Housekeeping**<br>Vijay Sharma, Todd Anderson, Wayne Parrott, Tracey Vieser |
| 11:20 a.m.–2:00 p.m. | **Session 1: Community Needs for Plant Transformation** |
| 11:15 – 11:20 a.m. | Introduction Session Chairs: Sally Assmann, William Gordon-Kamm |
| 11:20–11:30 a.m. | Lessons from Soybean Transformation<br>*Gary Stacey, University of Missouri* |
| 11:30–11:40 a.m. | *Populus* Recalcitrance to Transformation Presents Immediate Obstacles Toward Bioengineering Elite Bioenergy and Bioproducts Feedstocks<br>*Wellington Muchero, Oak Ridge National Laboratory* |
| 11:40–11:50 a.m. | Innovating Poplar: Tackling the Challenges of Genetic Transformation for Novel Traits<br>*Matias Kirst, University of Florida* |
| 11:50 a.m.–12:00 p.m. | Unveiling Sugarcane Genetic Engineering: Bitter Lessons Learned<br>*Hugo Molinari, SEMPRE AgTech* |
| 12:00–12:10 p.m. | Functional Genomic Tools in *Pancium* Grasses: Opportunities and Ongoing Challenges<br>*Tom Juenger, University of Texas* |
| 12:10–12:30 p.m. | Q&A |
| 12:30–1:45 p.m. | **Breakout Sessions**<br>Breakout Room A Leader: Sally Assmann<br>Breakout Room B Leader: William Gordon-Kamm<br>Breakout Room C Leader: Wayne Parrott<br>Breakout Room D Leader: Jeremy Schmutz |
| 1:45–2:00 p.m. | Report Out |

| | |
|---|---|
| 2:00–2:15 p.m. | **Break** |
| 2:15–3:30 p.m. | **Session 2: Current State and Challenges of Plant Transformation Facilities in Bioenergy Crops** |
| 2:15–2:20 p.m. | Introduction Session Chairs: Veena Veena, Jeremy Schmutz, Wayne Parrott |
| 2:20–2:30 p.m. | Challenges and Opportunities for Public Plant Transformation Facilities<br>*Kan Wang, Iowa State University* |
| 2:30–2:40 p.m. | Plant Transformation Services: Capacity, Efficiency, and Intellectual Property (IP) Considerations<br>*Veena Veena, Donald Danforth Plant Science Center* |
| 2:40–2:50 p.m. | Perspectives from a Director and User of Plant Transformation Services<br>*Joyce Van Eck, Boyce Thompson Institute* |
| 2:50–3:00 p.m. | Plant Transformation: A Public Sector Economy of Scale Model<br>*Thomas Clemente, University of Nebraska* |
| 3:00–3:10 p.m. | Levers to Reduce Bottlenecks for Making Transgenic Bioenergy Crops<br>*Alvar Carlson, Wisconsin Crop Innovation Center* |
| 3:10–3:25 p.m. | Q&A |
| 3:20–6:00 p.m. | **Session 3: Current State and Challenges of Plant Transformation in Bioenergy Crops** |
| 3:25–3:30 p.m. | Introduction Session Chairs: Veena Veena, Wayne Parrott, Jeremy Schmutz |
| 3:30–3:40 p.m. | Leveraging Oncogenes from a Shooty *Agrobacterium* Strain for Altruistic Transformation<br>*Greg Goralogia, Oregon State University* |
| 3:40–3:50 p.m. | Genomic Resources and Transformation Tools to Further the Development of Biomass Grasses<br>*Kankshita Swaminathan, HudsonAlpha Institute for Biotechnology* |
| 3:50–4:00 p.m. | Design and Assembly of Binary Vectors for Plant Transformation<br>*Laurens Pauwels, VIB-University of Ghent* |
| 4:00–4:10 p.m. | Bridging the Gap Between Sugarcane and Energycane Transformation<br>*Fredy Altpeter, University of Florida* |
| 4:10–4:20 p.m. | Q&A |
| 4:20–4:30 p.m. | **Break** |
| 4:30–5:45 p.m. | **Breakout Sessions**<br>Breakout Room A Leader: Jeremy Schmutz<br>Breakout Room B Leader: Margaret Young<br>Breakout Room C Leader: Veena Veena<br>Breakout Room D Leader: Wayne Parrott |
| 5:45–6:00 p.m. | Report Out |

## Tuesday, September 19

| | |
|---|---|
| 11:00–11:10 a.m. | **Introduction and Housekeeping by Session Chair** |
| 11:10 a.m.–1:40 p.m. | **Session 4: Developing an Inclusive Community and Pool of Talent** |
| 11:10–11:15 a.m. | Introduction Session Chairs: Margaret Young, Sally Assmann |
| 11:15–11:25 a.m. | Recruitment and Retention Challenges in Our Technical Training Programs<br>*Elizabeth (Betsy) Boedeker, St. Louis Community College* |
| 11:25–11:35 a.m. | Impact of Undergraduate Research Training: A Pathway for Graduate Programs in Plant Biotechnology<br>*Sarwan Dhir, Fort Valley State University* |
| 11:35–11:45 a.m. | Reshaping Approaches Culturally Relevant to Harness Diversity in the Training of the Next Generation of Bioenergy Minority Scientists<br>*Marceline Engin, Tuskegee University* |
| 11:45–11:55 a.m. | Practices and Lessons Learned from the NSF Advanced Technological Education (ATE) Program that Support the Bioeconomy Workforce<br>*V. Celeste Carter, National Science Foundation* |
| 11:55 a.m.–12:10 p.m. | Q&A |
| 12:10–1:20 p.m. | **Breakout Sessions**<br>Breakout Room A Leader: Sally Assmann<br>Breakout Room B Leader: Margaret Young<br>Breakout Room C Leader: Elizabeth (Betsy) Boedeker<br>Breakout Room D Leader: William Gordon-Kamm |
| 1:20–1:40 p.m. | Report Out |
| 1:40–2:00 p.m. | **Break** |
| 2:00–4:45 p.m. | **Session 5: Leveraging Existing and Future Genomics Tools to Develop New Tools and Technologies for Future** |
| 2:00–2:05 p.m. | Introduction Sessions Chairs: Jeremy Schmutz, Veena Veena, William Gordon-Kamm |
| 2:05–2:15 p.m. | Enabling Development of Plant Chassis for Sustainable Production of Biomolecules and Bioproducts<br>*Robin Buell, University of Georgia* |
| 2:15–2:25 p.m. | Developing Control Systems for Targeted Expression Engineering to Enable Plant Transformation<br>*Arjun Khakhar, Colorado State University* |
| 2:25–2:35 p.m. | Using Genomics to Identify Regulatory Networks Coordinating Somatic Regeneration<br>*Chris Saski, Clemson University* |
| 2:35–2:45 p.m. | Developing Synthetic Biology Tools to Improve Plant Engineering Efforts<br>*Patrick Shih, University of California–Berkeley* |

| | |
|---|---|
| 2:45–3:00 p.m. | Q&A |
| 3:00–4:30 p.m. | **Breakout Sessions**<br>Breakout Room A Leader: Jeremy Schmutz<br>Breakout Room B Leader: William Gordon-Kamm<br>Breakout Room C Leader: Veena Veena<br>Breakout Room D Leader: Wayne Parrott |
| 4:30–4:45 p.m. | Report Out |

## Wednesday, September 20

| | |
|---|---|
| 11:00–11:10 a.m. | **Introduction and Housekeeping by Session Chair** |
| 11:10–1:30 | **Session 6: Stewardship and Regulatory Landscape** |
| 11:10–11:15 a.m. | Introduction Session Chair: Wayne Parrott |
| 11:15–11:30 a.m. | Intellectual Property (IP) Considerations<br>*Allan Wenck, Syngenta* |
| 11:30–11:45 a.m. | Regulatory Barriers to Bioenergy Crop Adoption<br>*John Cordts, U.S. Department of Agriculture – Animal and Plant Health Inspection Service (Retired)* |
| 11:45 a.m.–12:00 p.m. | Key Aspects of Stewarding Energy Crops<br>*Ray Shillito, BASF (Retired)* |
| 12:00–12:10 p.m. | Q&A |
| 12:10–1:10 p.m. | **Breakout Sessions**<br>Breakout Room A Leader: Sally Assmann<br>Breakout Room B Leader: Margaret Young<br>Breakout Room C Leader: Veena Veena<br>Breakout Room D Leader: Wayne Parrott |
| 1:10–1:30 p.m. | Report Out |
| 1:30–1:50 p.m. | **Break** |
| 1:50–4:30 p.m. | **Session 7: New Methods for Gene Delivery, Transformation, and Regeneration (Open to All Crops)** |
| 1:50–1:55 p.m. | Introduction Session Chairs: William Gordon-Kamm, Veena Veena |
| 1:55–2:05 p.m. | Current Status and Potential Future Use of Morphogenic Genes in Recalcitrant Crops<br>*William Gordon-Kamm, Corteva* |
| 2:05–2:15 p.m. | Development of Virus-Based Delivery System for Transgene-Free Genome Editing in Plants<br>*SP Dinesh-Kumar, University of California Davis* |

| | |
|---|---|
| 2:15–2:25 p.m. | Non-Integrating Delivery of T-DNA to Plant Cells<br>*Stan Gelvin, Purdue University* |
| 2:25–2:35 p.m. | Tissue Culture-Free Transformation System for High Thoroughput Production of Transgenic Events<br>*Heng Zhong, Syngenta* |
| 2:35–2:45 p.m. | Q&A |
| 2:45–4:15 p.m. | **Breakout Sessions**<br>Breakout Room A Leader: William Gordon-Kamm<br>Breakout Room B Leader: Sally Assmann<br>Breakout Room C Leader: Veena Veena<br>Breakout Room D Leader: Jeremy Schmutz |
| 4:15–4:30 p.m. | Report Out |
| 4:30–4:45 p.m. | **Break** |
| 4:45–5:30 p.m. | **Workshop Report by Chair and Closing Remarks** |
| 5:30 p.m. | **Adjourn** |

# Appendix B
# Workshop Participants

## Chair

**Wayne Parrott**
*University of Georgia*

## Co-Chairs

**Sally Assmann**
*The Pennsylvania State University*

**William Gordon-Kamm**
*Corteva Agriscience*

**Jeremy Schmutz**
*HudsonAlpha Institute for Biotechnology*

**Veena Veena**
*Donald Danforth Plant Science Center*

**Margaret Young**
*Elizabeth City State University*

## Participants

**Samantha Abbad**
*SEMPRE AgTech*

**Amirhossein Ahkami**
*Environmental Molecular Sciences Laboratory*

**Fredy Altpeter**
*University of Florida*

**Cris Argueso**
*Colorado State University*

**Charles Armstrong**
*Plastomics*

**Fabricio Arraes**
*SEMPRE AgTech*

**Elizabeth (Betsy) Boedeker**
*St. Louis Community College*

**Federica Brandizzi**
*Michigan State University*

**Carol Robin Buell**
*University of Georgia*

**Edgar Cahoon**
*University of Nebraska–Lincoln*

**Alvar Carlson**
*Wisconsin Crop Innovation Center*

**V. Celeste Carter**
*National Science Foundation*

**Thomas Clemente**
*University of Nebraska–Lincoln*

**Sebastian Cocioba**
*Binomica Labs*

**John Cordts**
*U.S. Department of Agriculture (Retired)*

**Gözde Demirer**
*California Institute of Technology*

**Sarwan Dhir**
*Fort Valley State University*

**Savithramma Dinesh-Kumar**
*University of California–Davis*

**Timothy Donohue**
*Great Lakes Bioenergy Research Center*

**Marceline Egnin**
*Tuskegee University*

**Stanton Gelvin**
*Purdue University*

**Juan Pablo Giraldo**
*University of California–Riverside*

**Greg Goralogia**
*Oregon State University*

**Bjoern Hamberger**
*Michigan State University*

**Luis Herrera-Estrella**
*Texas Tech University*

**Todd Jones**
*Corteva Agriscience*

**Tom Juenger**
*University of Texas–Austin*

**Shawn Kaeppler**
*University of Wisconsin–Madison*

**Albert Kausch**
*University of Rhode Island*

**Arjun Khakhar**
*Colorado State University*

**Matias Kirst**
*University of Florida*

**Keunsub Lee**
*Iowa State University*

**Laurie Leonelli**
*University of Illinois Urbana–Champaign*

**Sally Mackenzie**
*The Pennsylvania State University*

**Pal Maliga**
*Rutgers University*

**Julie Mitchell**
*Oak Ridge National Laboratory*

**Lorena Moeller**
*Bayer Crop Science*

**Hugo Molinari**
*SEMPRE AgTech*

**Nigel Mouncey**
*DOE Joint Genome Institute*

**Wellington Muchero**
*Oak Ridge National Laboratory*

**Gloria Muday**
*Wake Forest University*

**Kiran Mysore**
*Oklahoma State University*

**Laurens Pauwels**
*VIB Crop Genome Engineering Facility*

**Yiping Qi**
*University of Maryland*

**Christopher Saski**
*Clemson University*

**Patrick Shih**
*Lawrence Berkeley National Laboratory*

**Ray Shillito**
*BASF (Retired)*

**Ashok Shrawat**
*Bayer Crop Science*

**Blake Simmons**
*Joint BioEnergy Institute*

**Rebecca Smith**
*University of Wisconsin*

**Vibha Srivastava**
*University of Arkansas*

**Bing Stacey**
*University of Missouri*

**Gary Stacey**
*University of Missouri*

**Kankshita Swaminathan**
*HudsonAlpha Institute for Biotechnology*

**Roger Thilmony**
*U.S. Department of Agriculture*

**James Thomson**
*U.S. Department of Agriculture*

**Chung-Jui (CJ) Tsai**
*University of Georgia*

**Jerry Tuskan**
*Center for Bioenergy Innovation*

**Joyce Van Eck**
*Cornell University*

**John Vogel**
*Lawrence Berkeley National Laboratory*

**Kan Wang**
*Iowa State University*

**Allan Wenck**
*Syngenta Crop Protection*

**Bing Yang**
*University of Missouri*

**Xiaohan Yang**
*Oak Ridge National Laboratory*

**Bradley Zamft**
*Google X*

**Feng Zhang**
*University of Minnesota–Twin Cities*

**Heng Zhong**
*Syngenta Crop Protection*

**Matthew Zinselmeier**
*Google X*

## U.S. Department of Energy Biological and Environmental Research Program

**Vijay Sharma, Organizer**

**Shing Kwok, Organizer**

**Kari Perez, Organizer**

**Todd Anderson**

**Dawn Adin**

**Resham Kulkarni**

**Ramana Madupu**

**Pablo Rabinowicz**

## Observers

**Dawn Carter**
*Rochester Institute of Technology*

**Karen Cone**
*National Science Foundation*

**John Erickson**
*National Institute of Food and Agriculture*

**Mary Fernandes**
*Solis Agrosciences*

**Stephen Herbert**
*U.S. Department of Energy, Basic Energy Sciences program*

**Susan Moser**
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**Diane Jofuku Okamuro**
*National Science Foundation, Plant Genome Research Program*

**Jack Okamuro**
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**Donna Pattison**
*University of Houston*

**Gerald Schoenknecht**
*National Science Foundation, Plant Genome Research Program*

**Christian Tobias**
*National Institute of Food and Agriculture*

**Clifford Weil**
*National Science Foundation*

# Appendix C
# Glossary

***Agrobacterium***

A genus of bacteria commonly used to transfer genetic material to plants

**allopolyploidization**

The process of forming a polyploid from two different parental species

**Assay for Transposase–Accessible Chromatin using sequencing (ATAC-seq)**

A rapid method for evaluating the accessibility of *chromatin* across the *epigenome*

***Bbm*/*Wus* (Baby Boom/Wuschel)**

Two genes that are able to stimulate regeneration and produce high transformation frequencies

**bioeconomy**

The infrastructure, innovation, products, technology, and data derived from living organisms and biological information derived from them to drive economic growth, improve public health and agriculture, and provide security benefits

**biotechnology**

A set of biological techniques developed through basic research that provide organisms with new characteristics. In particular, biotechnology refers to the use of *recombinant DNA*, cell fusion, and bioprocessing techniques

**breed true**

To produce offspring whose genotypes and phenotypes are identical to the parents' genotypes and phenotypes

**callus**

Undifferentiated unorganized mass of plant cells in culture

**cassette**

A DNA sequence containing one or more chimeric genes, and having particular restriction sequences at both ends, to facilitate its insertion into a vector

**chromatin**

A complex of DNA and protein that makes up the chromosomes in eukaryotic cells

***cis*-regulatory elements**

Collections of transcription-factor binding sites and other noncoding DNA sequences (typically within enhancers and promoters) that regulate gene expression

**cisgenic plant**[1]

Genetically engineered plant with a gene from a sexually compatible species; that is, an event that could be accomplished through conventional plant breeding

**conspecific**

Belonging to the same species

**construct**

A particular recombinant DNA molecule which has been designed to be engineered into plants, especially when used to test a specific sequence for function

**conventional plant breeding**[2]

Modification of the genetic constitution of a plant through sexually crossing different genomes or mutagenizing a plant's genome with chemical methods or irradiation, and selecting desirable plants to serve as parent lines

**CRISPR/Cas**

Adaptive immune system in bacteria and archaea used in genome editing to insert a target gene into a genome at a precise location identified by a unique basepair sequence in the DNA

**cotyledon**

An embryonic seed leaf; characteristically one for monocots and two for dicots

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[1,2] Used with permission of the National Academies Press from *Genetically Engineered Crops: Experiences and Prospects* the National Academies of Sciences, Engineering, and Medicine. Copyright 2016. Permission conveyed through Copyright Clearance Center, Inc.

**cultivar**[3]

A variety of a plant species with distinct genetically based morphological, physiological, cytological, or chemical characteristics, produced and maintained by cultivation

***de novo***

From the beginning

**dicot**

Flowering plant with two *cotyledons* (i.e., embryonic seed leaves)

**diploid**

An organism that has two full sets of genetic material consisting of paired chromosomes, one from each parental set

**DNA affinity purification sequencing (DAP-seq)**

A high-throughput technique for identifying genome-wide transcription factor binding sites that couples *in vitro* expression of transcription factors with next-generation sequencing of a genomic DNA library

**DNA construct**

An artificially created piece of DNA designed to be introduced into a cell. These are components of cassettes. Also, *multigenic constructs:* containing multiple genes

**endonuclease**

A protein that recognizes specific, short nucleotide sequences and cuts nucleic acid polymers at those sites

**epigenome**

The chemical additions that modify, or mark, DNA within a genome in a way that tells it what to do, where to do it, and when to do it

**epigenetics**

The study of how gene expression can change without the genes themselves changing

**event**

A plant produced from each unique change in a genome obtained with recombinant DNA

**explant**

Living tissue removed from an organism and placed in a medium for tissue culture

**floral dip**

A method for plant transformation in arabidopsis and a few other plant species where flower buds are dipped in a suspension of *Agrobacterium* that colonize the interior of developing ovaries and transform female gametophyte cell lineages. Transgenic seedlings are then produced from the transformed seeds

**functional genomics**

The study of the functions and interactions of genes and proteins

**gene**

The fundamental physical and functional unit of heredity. A gene is an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a specific functional product (i.e., a protein or RNA molecule)

**genetic engineering**

Altering the genetic material of cells or organisms through addition of DNA to enable them to make new substances or perform new functions

**genetics**

The study of genes and their roles in inheritance (i.e., the way that certain traits or conditions are passed down from one generation to another)

**genomics**

An interdisciplinary field of science that focuses on the structure, function, evolution, mapping, and editing of genomes

**genotype**

An organism's genetic constitution

**germplasm**[4]

Seeds, tissues, or plants that represent the genetic diversity of a species maintained for breeding, research, and conservation efforts

**guide RNA (gRNA)**

A specific RNA sequence that recognizes the target DNA region of interest (and directs the Cas nuclease there for editing)

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**haplotype**

A chromosomal segment identifiable by its genetic variants that tends to be inherited as a unit

**homoeologous**

Relating to gene pairs that originated by speciation and then were reunited in the same genome by *allopolyploidization*

**homology-dependent repair**

Naturally occurring mechanism for repairing double-stranded DNA breaks that uses as a template for repair either the DNA from a homologous chromosome or an artificially added DNA sequence that is homologous to the broken DNA

***in planta***

Within a living, intact plant

**intragenic**

Within a gene; a transgenic plant made by using a gene from that same plant

**introgression**

The movement of genes from one species or population into the gene pool of another through hybridization and backcrossing

**knockout**

Deactivation of a specific gene or genes

**landing pad**

One or more copies of designed sequence inserted into a genome of interest that are used to stably and precisely integrate one or multiple genes into the genome

**metabolomics**

The characterization of metabolites, small molecules, and their intermediates, and the processes by which they are produced and consumed during cell metabolism

**monocot**

Flowering plant with one *cotyledon* (i.e., embryonic seed leaf)

**morphogenic**

Relating to the origin and development of plant organs, such as embryos, leaflets, or meristems

**mutagenesis**

Process by which an organism's DNA changes

**neo-functionalization**

An adaptive process where one gene copy mutates into a function not present in the original gene

**nonhomologous end-joining**

Naturally occurring mechanism in which DNA molecules with double strand breaks are repaired

**nonrecombinant techniques**

Methods for altering plant DNA that do not involve introducing foreign DNA

**obligate outcrossers**

Plants that are self-incompatible and so cannot self-fertilize to produce seed

**paralogs**

Genes that arise from gene duplication events of individual genes within a genome

**phenology**

Study of the timing of lifecycle events at the population level, including flowering, fruit production, and leaf fall

**phenotype**

Physical characteristics of an organism

**polyploid**

Containing more than two sets of chromosomes

**propagule**

Plant material that can give rise to a new plant

**promoters**

A region of DNA upstream of a gene where relevant proteins bind to initiate or repress transcription of that gene

**proteomics**

The study of all the proteins in a cell, tissue, or organism

**protoplast**

Plant cell with its cell wall removed

**pseudogenization**

The loss of genes through mutation or copy failures, resulting in a DNA sequence similar to the original gene but without the ability to form functional proteins

**quality**

In random integration, quality refers to engineered plants that have only one copy of the intact transgene in their genome and in a location that does not affect other genes. For CRISPR-based approaches, quality refers to generation of the desired edit(s) and production of transgene-free progeny

**recombinant DNA (rDNA)**

An artificially formed combination of DNA

**recalcitrance**

A plant's inability to regenerate from cell or tissue culture, which in turn impedes its capacity for transformation

**regeneration**

The process of producing an entire plant from individual cells

**rhizome**

An underground plant stem that sends out roots and shoots capable of producing a new plant

**safe harbors**

In genome engineering, regions of the genome that can accommodate transgenic insertion without disrupting the function of host cells

**single-guide RNA (sgRNA)**

A short sequence of RNA that directs Cas proteins where to cut a DNA sequence for genome editing

**subfunctionalization**

Evolution whereby each duplicated copy of a gene takes on separate functions by retaining part of its original function

**subgenome**

Each parental genome within an allopolyploid

**suckering**

A form of asexual reproduction where new plants arise from a bud off a root or stem

**synthetic biology**

The redesigning of organisms for useful purposes by engineering them to have new properties

**T0 generation**

First generation of plants following genetic modification and regeneration

**tetraploid**

Containing four copies of each chromosome (double the DNA of a diploid)

**tissue culture**

Growth of cells or tissues in an artificial medium

**transcription factor**

Protein that binds to regulatory regions and helps control gene expression.

**transcriptomics**

The identification and study of the transcriptome, or complete set of RNA molecules expressed in a cell, tissue, or organism

**transformation**

The process of introducing DNA into cells or tissues

**transformation efficiency**

The number of events recovered per *explant* or per unit of time and labor

**transgenic**

An experimentally produced organism in which DNA has been artificially introduced and incorporated into it

**vector**

A self-replicating, circular piece of DNA into which another DNA fragment can be integrated, and used to amplify the DNA fragment or used to introduce it into a target cell

# Appendix D
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# Appendix E
# Acronyms and Abbreviations

| | |
|---|---|
| **AI** | artificial intelligence |
| **APHIS** | Animal and Plant Health Inspection Service |
| **ATAC-Seq** | assay for transposase-accessible chromatin with sequencing |
| **BER** | Biological and Environmental Research program |
| **CRE** | Cis-regulatory elements |
| **CUREs** | course-based Undergraduate Research Experiences |
| **DAP-Seq** | DNA affinity purification sequencing |
| **DOE** | U.S. Department of Energy |
| **EPA** | U.S. Environmental Protection Agency |
| **FDA** | U.S. Food and Drug Administration |
| **gRNA** | guide RNA |
| **HDR** | homology-directed repair |
| **IBC** | Institutional Biosafety Committee |
| **IP** | intellectual property |
| **ML** | machine learning |
| **NHEJ** | nonhomologous end joining |
| **NIH** | National Institutes of Health |
| **NSF** | National Science Foundation |
| **PCR** | polymerase chain reaction |
| **PIPRA** | Public Intellectual Property Resource for Agriculture |
| **RCN** | Research Coordination Network |
| **REU** | Research Experiences for Undergraduates |
| **scATAC-seq** | single cell assay for transposible-accessible chromatin |
| **sgRNA** | single guide RNA |
| **USDA** | U.S. Department of Agriculture |