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# Advancing Crop Transformation in the Era of Genome Editing <sup>OPEN</sup>

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**Plant transformation has enabled fundamental insights into plant biology and revolutionized commercial agriculture. Unfortunately, for most crops, transformation and regeneration remain arduous even after more than 30 years of technological advances. Genome editing provides novel opportunities to enhance crop productivity but relies on genetic transformation and plant regeneration, which are bottlenecks in the process. Here, we review the state of plant transformation and point to innovations needed to enable genome editing in crops. Plant tissue culture methods need optimization and simplification for efficiency and minimization of time in culture. Currently, specialized facilities exist for crop transformation. Single-cell and robotic techniques should be developed for high-throughput genomic screens. Plant genes involved in developmental reprogramming, wound response, and/or homologous recombination should be used to boost the recovery of transformed plants. Engineering universal *Agrobacterium tumefaciens* strains and recruiting other microbes, such as *Ensifer* or *Rhizobium*, could facilitate delivery of DNA and proteins into plant cells. Synthetic biology should be employed for de novo design of transformation systems. Genome editing is a potential game-changer in crop genetics when plant transformation systems are optimized.**

## INTRODUCTION

We face the critical challenge of producing sufficient food for a growing human population living in a changing and unstable climate. Substantial public research investments have been made to sequence, assemble, and characterize the genomes of major crop plants. This investment in plant science has enabled foundational discoveries of crop genes and their functions. This

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knowledge is poised to be leveraged for increased agricultural production by using synthetic biology, including tools for precise plant breeding. Genome editing is an unprecedented technological breakthrough, yet there are bottlenecks to its implementation for crop improvement. Defining gene sequences from diverse species and cultivars has far outpaced our ability to alter those genes in crops. A major challenge in plant genetic and genome editing, and future plant breeding is our ability to rapidly manipulate plant genomes via genetic transformation (Figure 1).

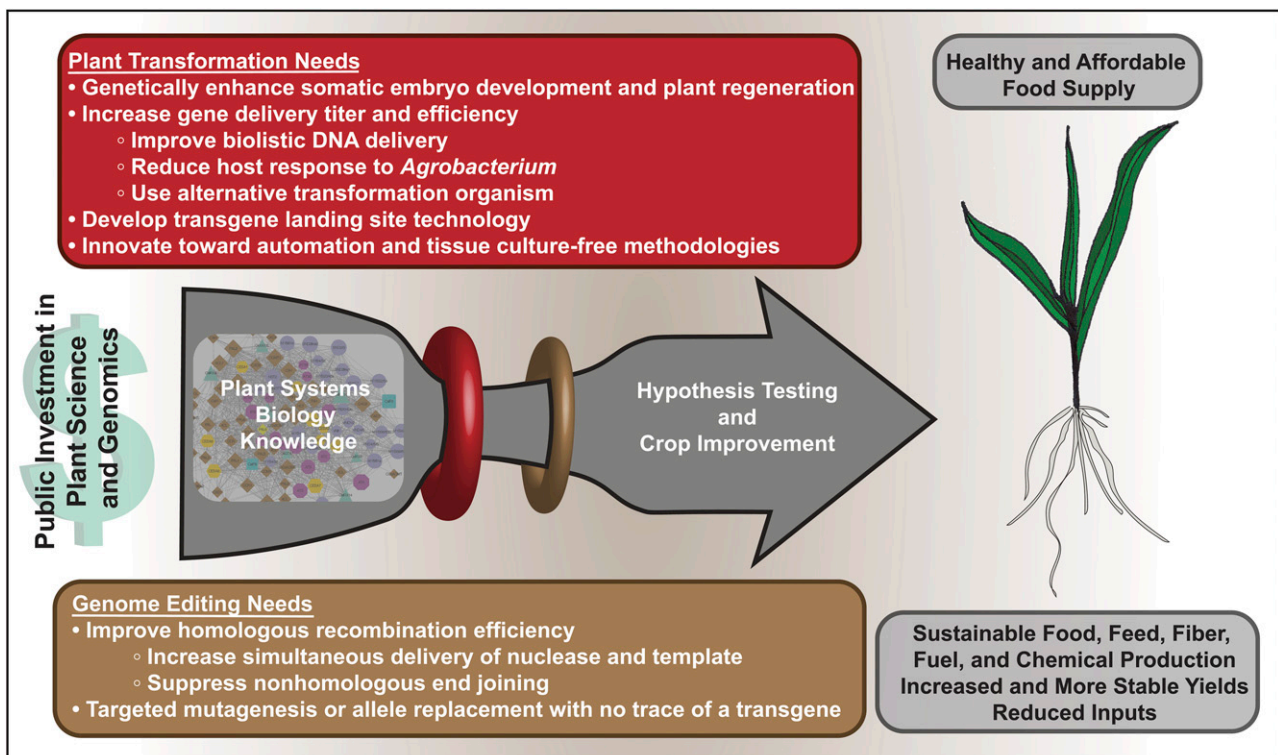
Plant transformation encompasses two distinct consecutive steps: (1) DNA introduction into plant cells (sometimes known as transient transformation, in which transgenes have not yet integrated into the genome), and (2) integration of the introduced DNA into the plant genome (stable transformation). Each step is useful in basic plant research and biotechnology, but the second step is necessary to produce transgenic plants with heritable traits of interest. For most crops, transgenic plant production requires the ability to regenerate plants from transformed tissues. Although considered part of the transformation process, the regeneration step is often a greater bottleneck than is the stable integration of DNA sequences (Figure 2). In this article, we review current knowledge and bottlenecks to plant transformation and the

implementation of high-throughput genome editing. As we look to the future, we propose strategies to address these shortcomings.

### INCREASED PLANT TRANSFORMATION DEMAND FOR GENOME EDITING

As originally performed, plant transformation results in random integration of new sequences into plant genomes. Remarkable advances over the past 15 years now provide more control over integration and permit precise, targeted modifications to DNA sequences in plant cells (genome editing) (Voytas and Gao, 2014; Baltes and Voytas, 2015). Genome editing uses customizable, sequence-specific nucleases (SSNs) that generate a DNA double-strand break (DSB) at a specific genomic target. These sites allow targeted mutagenesis or specific editing depending on how the cell repairs the break.

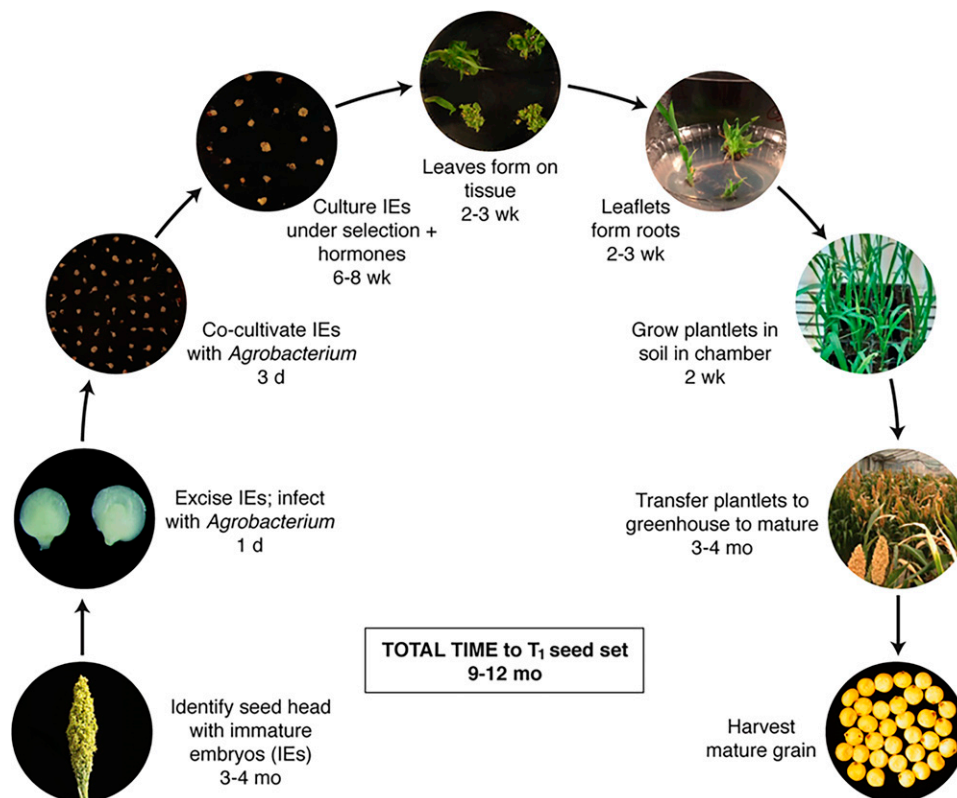
The most common cellular mechanism of break repair in angiosperms is nonhomologous end joining (NHEJ). This pathway often results in small changes at the repaired site and can be used to perform targeted mutagenesis to alter gene expression or function (Puchta, 2005; Wang et al., 2014; Li et al., 2012). To achieve targeted mutagenesis, SSNs are either transiently



**Figure 1.** Current Bottlenecks in Applying Genome Editing to Crop Functional Genomics and Crop Improvement.

The main bottleneck is in plant transformation and regeneration. A secondary bottleneck is in the delivery of genome editing reagents to plant cells to produce the intended effects.

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**Figure 2.** Sorghum (*Sorghum bicolor*) Is a Crop Recalcitrant to Transformation and Regeneration.

Starting left and proceeding clockwise are representations of steps and time required for each step in the method, from growth of donor plants to provide target immature embryos to the harvesting of mature seed. Times required at each step is indicated as d (days), wk (weeks), and mo (months). Similar protocols and timelines prevent the high-throughput transformation and genome editing for most important U.S. crops.

delivered to protoplasts or stably incorporated into the genome as a transgene. In the latter case, during transgenic plant growth, at some frequency the SSN mutates the lineages later incorporated into reproductive cells, enabling mutations to be transmitted to progeny. In subsequent generations, the nuclease transgene can be segregated away, to obtain a nontransgenic plant with mutations in the target locus of interest.

Cells can also repair DSB sites by homologous recombination (HR), in which a template—either a homologous chromosome or a user-supplied sequence—is used for repair (Voytas and Gao, 2014; Baltes and Voytas, 2015). A user-supplied repair template is provided exogenously along with the SSN and can contain specific genome edits, ranging from single base changes that alter a proteins' amino acid sequence, to multiple transgenes that become incorporated at the break site. A challenge for HR-mediated gene editing is that it requires simultaneous delivery of both the SSN and the repair template. Furthermore, repair through NHEJ predominates in somatic cells and competes with the HR pathway. To increase the frequency of HR, virus-based vectors are being developed that increase the SSN titer and

repair templates delivered to the cell (Baltes et al., 2014). Likewise, biolistic gene transfer may be superior to *Agrobacterium tumefaciens*-mediated gene transfer for HR-mediated gene editing (Svitashev et al., 2015; Sun et al., 2016) by providing larger quantities of repair template and high levels of transient expression. Suppression of core components of the NHEJ pathway can also be used to increase frequencies of HR (Qi et al., 2013).

A key technical advance in gene editing has been the development of reagents that make targeted DSBs with high specificity in complex genomes. The first such reagent platforms—meganucleases, zinc finger nucleases, and TAL effector nucleases—used engineered DNA binding proteins to recognize target DNA sequences (Voytas and Gao, 2014; Baltes and Voytas, 2015) and therefore required protein engineering. The advent of CRISPR/Cas and related reagents, which use guide RNAs that recognize target DNA sequences through Watson-Crick base pairing, dramatically simplified reagent design (Jinek et al., 2012; Cong et al., 2013; Mali et al., 2013). Thus, the deployment of CRISPR/Cas has made genome editing easily accessible, leading to broad adoption and rapid innovation. Below, we review research findings and

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propose strategies to more fully implement genome editing for functional genomics research and crop improvement.

### IMPROVING PLANT TRANSFORMATION TECHNOLOGIES

Agrobacterium- and particle bombardment-mediated transformation have been practiced for more than three decades (Figure 3). However, current approaches remain inefficient for many crops. The primary challenges include (1) long tissue culture periods required to recover transgenic plants from engineered cells and tissues (Figure 2), (2) low frequency of stably transformed events, (3) low DNA titers delivered by Agrobacterium-mediated gene transfer, which are insufficient to drive HR, and (4) low precision of bombardment-mediated gene transfer. Each challenge represents a suite of scientific and engineering problems that, when solved, would significantly reduce time and labor for crop engineering. For example, the floral dip transformation protocol of *Arabidopsis thaliana* has been a game-changing technology for that species, not because it increased transformation frequency but because it eliminated the need for tissue culture to recover transformed plants. Furthermore, this method is so technically simple to perform that even entry-level researchers can successfully transform *Arabidopsis*. The ideal solution for crop plants would be the discovery of simplified protocols for transformation that do not require tissue culture and could be utilized in many labs. However, there is no clear path to developing such technologies, and it is important to continue to improve tissue culture-based protocols that are widely used to engineer crop plants. Indeed, because of the challenges described above, in the early 2000s several dedicated plant transformation facilities were founded in the US to enable crop transformation services (Supplemental Table 1). They are integral to the discussion of crop genome editing throughput given the possible paths crop transformation might take in the future (Supplemental Table 1). In the following sections, we highlight different aspects of plant transformation that are potential targets for improvement.

#### Increasing Transformation Efficiency in Crops: Improving Existing Platforms

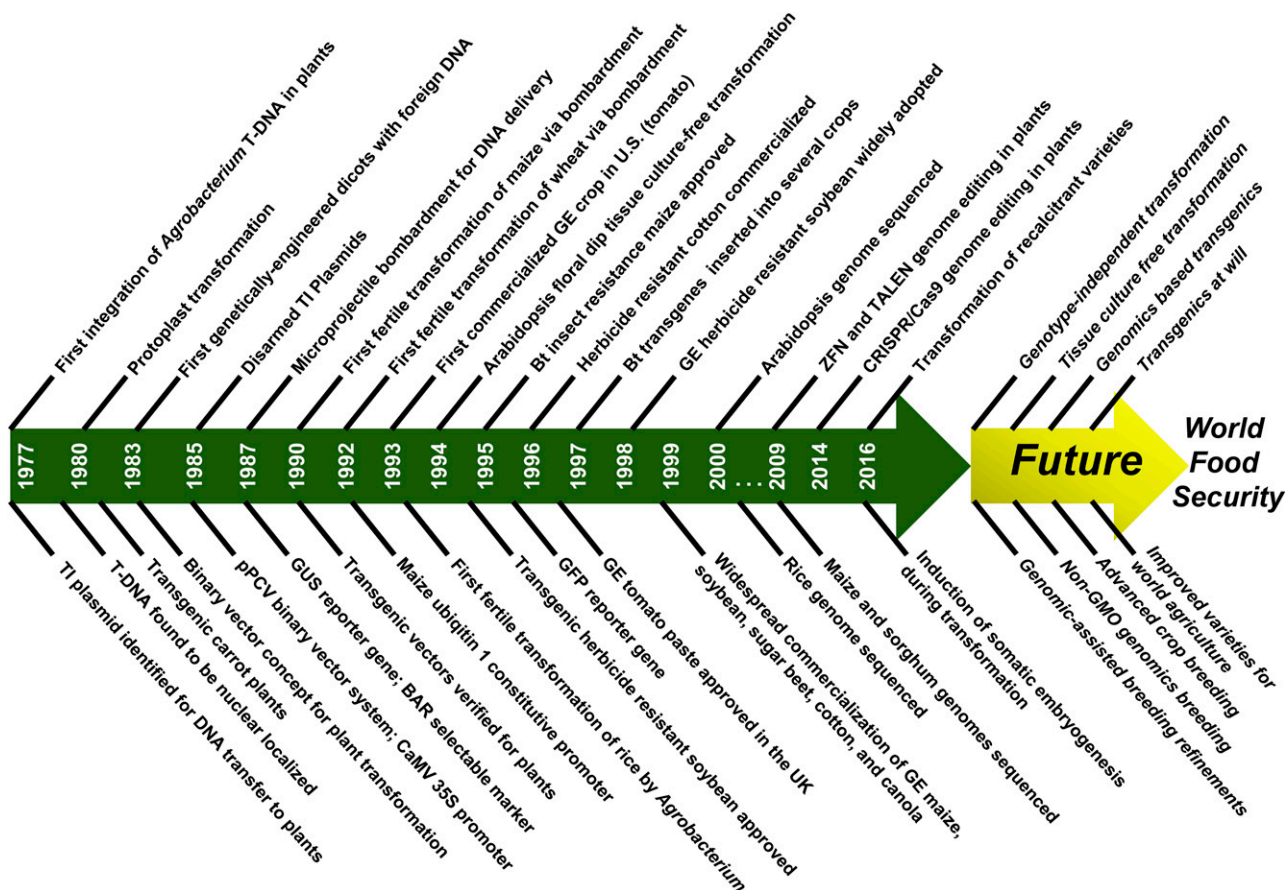
Recalcitrance to tissue culture and transformation limits efforts to use transgenesis and genome editing for crop functional genomics research (Shrawat and Lörz, 2006; Hiei et al., 2014). Efficient Agrobacterium-mediated transformation is typically limited to a narrow range of genotypes within a species (Nam et al., 1997). Often, cells that are readily transformed cannot be regenerated, and vice versa. Biolistic gene transfer can be applied to a wider range of genotypes than Agrobacterium-mediated gene transfer (Altpeter et al., 2005) but can be limited by the inability to regenerate plants after bombardment. Regeneration response and transgene performance following biolistic gene transfer depend on particle type, size, quantity and acceleration, DNA amount and structure during particle coating, tissue type, and pretreatment (Klein et al., 1988; Vain et al., 1993; Kausch et al.,

1995; Frame et al., 2000; Fu et al., 2000; Popelka et al., 2003; Sandhu and Altpeter 2008; Lowe et al., 2009; Sivamani et al., 2009; Wu et al., 2015). Therefore, there is a need for the development of alternative nano- or microparticles, target tissues, and particle coating and delivery protocols for biolistic gene transfer. Improvements in each of these areas should enhance delivery of intact single-copy expression cassettes while reducing tissue damage. Further research also is required to enhance regeneration and transformation responses of a wide range of target tissues and genotypes.

There are several potential approaches to optimize cell and tissue culture. Traditionally, callus, somatic embryos, and other tissues harboring totipotent cells have been used for bombardment or Agrobacterium-mediated transformation. In most cases, manipulation of plant developmental programs *in vitro* has been accomplished with exogenous application of plant growth regulators, namely, auxins and cytokinins. The choice of growth regulators and their sequence and timing of exposure are currently determined empirically for each species and often adjusted for each genotype. The molecular mechanisms for induction of cultured tissues from somatic cells are becoming better understood, and stress plays a striking role in this process (Florentin et al., 2013; Ikeuchi et al., 2013; Fehér, 2015; Grafi and Barack, 2015). Genetic and epigenetic mechanisms appear to control callus formation and the redifferentiation of organs and somatic embryos from different tissues through modulating hormonal signaling involving AUXIN RESPONSE FACTORS (Fan et al., 2012); cytokinin type-B ARABIDOPSIS RESPONSE REGULATORS (Sakai et al., 2001; Tajima et al., 2004); transcription factors, such as LEAFY COTYLEDON1 (LEC1), WUSCHEL (WUS), and BABY BOOM (ODP2); AGAMOUS-LIKE15; and the SOMATIC EMBRYOGENESIS RECEPTOR KINASE (reviewed in Ikeuchi et al., 2013; Fehér, 2015). The RECEPTOR-LIKE PROTEIN KINASE1, an abscisic acid-related receptor, appears to be important for the regeneration capacity of calli induced in *Arabidopsis* roots (Motte et al., 2014). Epigenetic regulation (chromatin remodeling) through DNA methylation and histone modification directly affect the expression of many of the key regulators of cell proliferation and differentiation (Zhao et al., 2001; Furuta et al., 2011; Florentin et al., 2013). Surprisingly, altering endogenous changes in plant developmental biology via genetic manipulation is an underutilized approach. In this respect, tuning the expression of regulatory factors such as LEC1, WUS, and ODP2 has been used to reprogram transformed cells, induce somatic embryogenesis, and increase regeneration frequency of transgenic plants (Lotan et al., 1998; Boutilier et al., 2002; Zuo et al., 2002a, 2002b; Bouchabké-Coussa et al., 2013; Florez et al., 2015). Regulated expression of such factors should be useful for crop engineering.

In addition, deploying single-cell techniques in plants would be valuable for high-throughput screening and transgenic combinatorial approaches. Protoplasts have long been used as a tool for plant molecular biology. Recently, a plant transformation and genome editing robot was developed for transfection and screening of plant protoplasts (Dlugosz et al., 2016). It is possible

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**Figure 3.** Important Historical Milestones in Plant Transformation.

Since its beginning in 1977, the pace of crop transformation technology development has not been linear. In recent years, the genome editing revolution begs for crop transformation improvements to enable greater food security.

that automation would enable large-scale screens such as those performed in the recent work by Wang et al. (2015) in which CRISPR-mediated mutations were used to determine essential genes required for human cell proliferation. Using an automated cell screen, every gene could be knocked out sequentially in crop cells for a massive functional analysis.

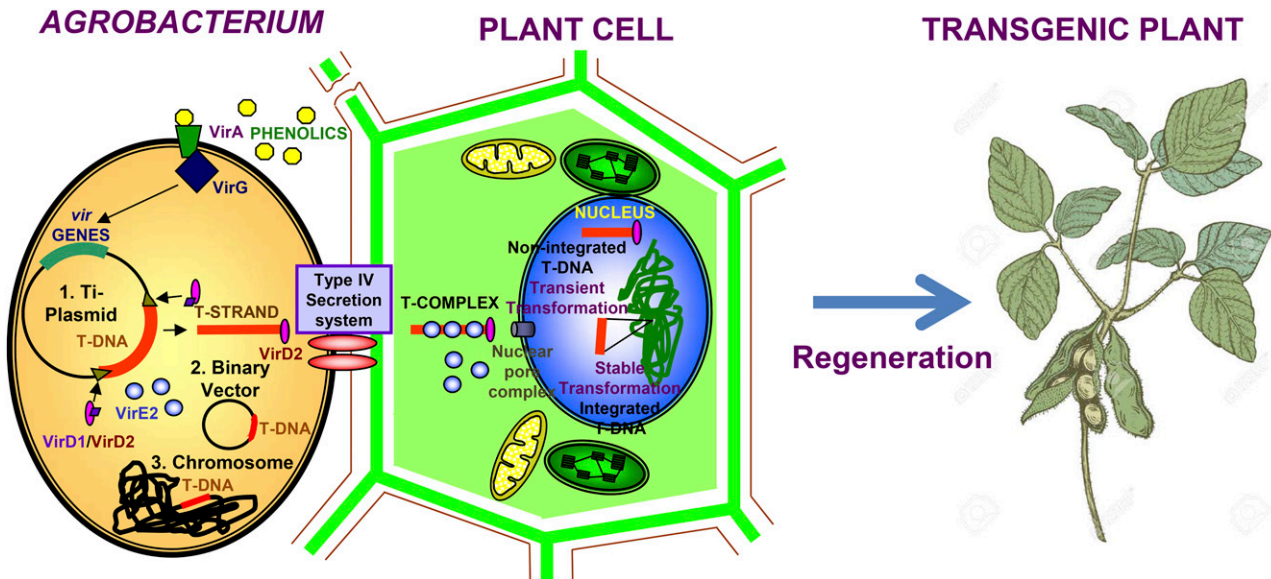
Agrobacterium-mediated transformation consists of bacterial attachment, T-DNA and virulence (*vir*) effector protein transfer, cytoplasmic trafficking of T-DNA/protein complexes, nuclear entry, removal of proteins from the T-strand, T-DNA integration, and transgene expression. We have a basic understanding of the plant and bacterial virulence proteins that are important for these processes (Figure 4; Gelvin, 2012; Magori and Citovsky, 2012; Lacroix and Citovsky, 2013). For example, altered production of the plant proteins has increased host susceptibility to transformation (Gelvin, 2010). In particular, an *Arabidopsis* MYB transcription factor (*MTF*) appears to function as a global negative regulator of transformation susceptibility; downregulation of *MTF* can increase *Arabidopsis*

transformation 15-fold (Sardesai et al., 2013, 2014). Conversely, some host proteins are activated or produced in response to *Agrobacterium*. The bacterium likely subverts these proteins to facilitate infection (Zaltsman et al., 2010). Thus, it is likely that priming the host plant by downregulation of one or more of its infection-responsive genes could enhance *Agrobacterium*-mediated transformation.

Plant tissue browning and necrosis in response to *Agrobacterium* infection reduces transformation frequency. Antioxidants in the infection medium can attenuate this reaction, but plant cells may still respond to the *Agrobacterium* pathogen-associated molecular pattern Ef-Tu (Zipfel et al., 2006) and perhaps bacterial surface molecules. Research is needed to identify bacterial-associated molecules that induce localized defense responses in crop plants and either eliminate or mask them, generating a “stealth *Agrobacterium*” strain that does not elicit necrotic responses.

Particular combinations of *Agrobacterium* *vir* genes and bacterial chromosomal backgrounds influence virulence on different

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**Figure 4.** Overview of Agrobacterium-Mediated Transformation to Generate a Transgenic Plant.

Phenolic compounds secreted by wounded plants are perceived by the Agrobacterium VirA/VirG two-component sensing system, resulting in induction of virulence (*vir*) genes. Among these genes, *virD1* and *virD2* form a site-specific nuclease that nicks the T-DNA region at border sequences. In nature, T-DNA resides on the Ti-(tumor inducing) or Ri-(root inducing) plasmid (1), but in the laboratory, T-DNA can be “launched” from binary vectors (2) or from the bacterial chromosome (3). VirD2 covalently links to single-strand T-DNA and leads T-strands through a Type IV secretion system (composed of VirB and VirD4 proteins) into the plant. Other transferred virulence effector proteins are VirE2 (a single-strand DNA binding protein proposed to coat T-strands in the plant cell) and VirD5, VirE3, and VirF (not pictured). Within the plant, VirD2/T-strands likely form complexes with VirE2, other Vir effector proteins, and plant proteins. These complexes target the nucleus. Once inside the nucleus, proteins must be stripped from T-strands, which can replicate to a double-strand nonintegrated form (transient transformation). T-DNA can integrate into the plant chromosomes, resulting in stably transformed cells. These cells can be regenerated to plants harboring and expressing transgenes.

plant species (Hood et al., 1987). Future studies should include rigorous analysis of combinations of these factors to generate Agrobacterium strains with a broader host range. Because transfer of small RNA molecules from some pathogens to plants affects virulence (Weiberg et al., 2013; Weiberg and Jin, 2015), research should also be conducted to determine if similar RNA transfer occurs during Agrobacterium infection and whether manipulation of such transfer can enhance transformation.

### Producing High-Quality Transgenic Events

The generation of single-copy transgenic events, especially when inserted at a predetermined locus to allow appropriately regulated levels of expression, is beneficial for commercialization of engineered crops. Currently, a “numbers game” approach is used to produce many events with random transgene insertions, and the “best” events are selected, screened, and evaluated over many subsequent generations. A more rational approach is needed. Transgene expression also needs to be stable across environments, including biotic and abiotic stress conditions encountered in agricultural fields. Even for readily transformed species, transgene expression can be unpredictable and unstable (Brandle

et al., 1995; Ni et al., 1995; Henneberry et al., 2000). Transgene expression does not always correlate with transgene copy number and can vary greatly among single-copy integration events. The only known difference among these transgenic events is integration site; therefore, expression differences have been attributed to “position effects” (Elmayan and Vaucheret, 1996; Mlynarova et al., 1996). Thus, it is desirable to target transgenes to genomic sites where expression would be predictable. These sites should be outside any gene or intergenic region important for plant growth, yield, nutrition, or other physiological traits. Another factor that figures in the selection of “safe spots” for landing transgenes is the avoidance of recombination hot spots and loci that may favor introgression into wild relatives of the crop (Stewart et al., 2003). Research is needed to compare genome editing systems (such as CRISPR, TAL effector nuclease, and zinc finger nuclease; Baltes and Voytas, 2015) to each other and to site-specific recombination systems (such as Cre/lox or Flp/rtt; Srivastava and Thomson, 2016). These studies should evaluate the frequency of unintended random integration events and develop protocols to rapidly identify, suppress, or segregate such events.

In addition, research is needed to optimize protocols that eliminate unwanted integration of the transformation vector DNA

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backbone into plant genomes. In particle bombardment, this is accomplished by physically separating the vector backbones from the expression cassettes. In *Agrobacterium*-mediated transformation, one method to minimize vector backbone integration is to “launch” T-DNA from the *Agrobacterium* chromosome (Lee et al., 2001; Oltmanns et al., 2010). However, this technology needs further development to produce high-quality transformation events without decreasing plant transformation frequency.

### NOVEL APPROACHES FOR TRANSFORMATION AND GENOME EDITING TO ENABLE CROP GENOMICS

Beyond improvements in existing plant transformation methodologies, higher throughput and novel transformation approaches could dramatically enhance plant genomics research. We will review relevant literature and speculate about the most promising technologies that could affect transformability. While casting into the future is far from certain with regards to which particular techniques will emerge as winners, the authors agree that relying solely on improvements in 30-year-old technologies is insufficient. Successful development of new methodologies should accelerate our understanding of the plant genes underlying crop productivity.

#### Introduction of Other Biologically Active Molecules into Plant Cells

*Agrobacterium* uses a type IV secretion system (T4SS) to deliver virulence effector proteins to plant cells (Cascales and Christie, 2004; Alvarez-Martinez and Christie, 2009). One of these proteins, VirD2, is covalently linked to single-strand T-DNA (T-strands; Vogel and Das, 1992; Ward and Barnes, 1988; Young and Nester, 1988), thus permitting T-DNA transfer through a protein transfer apparatus. We consider it feasible to deliver other protein- and nucleic acid molecules into cells and to optimize codelivery of nonintegrative DNA, functional RNA, and protein to avoid transgene integration and support genome editing via homology-dependent repair (HDR). A T4SS or T6SS already present in many *Agrobacterium* strains could be used (Wu et al., 2012; Lin et al., 2013). Sequenced strains of *Agrobacterium* do not possess a T3SS such as those used for transferring effector proteins from other bacterial pathogens to plants (Büttner and He, 2009). Adding genes encoding a T3SS to *Agrobacterium* would provide it with a route for protein transfer that would not compete with endogenous T4SS.

Viral and cell free systems could also be instrumental for the introduction of molecules into plant cells to optimize the codelivery of proteins and RNA molecules with or without the use of nanoparticles, cell-penetrating peptides, and/or lipid vesicles. Optimization is needed for codelivery of single-strand DNA templates, functional RNA, and/or proteins such as viral replicases to prevent transgene integration and support genome editing tools via HDR.

#### DNA Transfer and Gene Expression in the Absence of Integration

DNA transfer to plant cells usually involves transgene integration into the host genome. By contrast, the introduction of genes without subsequent integration is important for HDR, transient expression of genome-editing tools, and transient expression of genes important for developmental reprogramming during regeneration. To eliminate integration, we need to better understand how *Agrobacterium* integrates T-DNA into plant genomes. Both *Agrobacterium* and plant genes are important for T-DNA integration (Gelvin, 2010), but we have an incomplete understanding of how to manipulate those genes to prevent integration. An *Agrobacterium* strain harboring a mutant VirD2 protein is mildly deficient in transient T-DNA delivery but severely deficient in T-DNA integration (Narasimhulu et al., 1996; Mysore et al., 1998). Thus, a nonintegrating synthetic VirD2 with optimal transient expression properties might be developed for efficient delivery of T-DNA without integration.

For particle bombardment, functionalized gold nanocomposites (Li et al., 2009) or a particle coating chemistry that prevents DNA release into the cell nucleus could facilitate transient nuclear expression without transgene integration. Bombardment of single-stranded DNA has been used as a strategy to avoid template integration during HDR-mediated genome editing (Svitashev et al., 2015). However, the template design and delivery specifications require optimization for reproducibility across different species (Sun et al., 2016).

Transient expression systems could be favored by the adaptation of selectable markers and reporter genes for counterselection against integration events. Templates and genome editing tools could also be designed for self-excision of randomly integrated events. DNA-free genome editing (Woo et al., 2015) is a foreseeable approach for genomics research and advanced plant breeding. In this case, *in vitro*-translated Cas9 in a complex with guide RNA was transfected into plant protoplasts and non-transgenic genome-edited plants were regenerated (Woo et al., 2015). Plant breeders often desire specific mutations in a DNA sequence without an accompanying transgenic footprint in the genome. Therefore, DNA-free genome editing approaches are attractive on many levels.

#### Development of Non-*Agrobacterium* Biological Systems to Deliver DNA and Proteins into Plant Cells

Although *Agrobacterium*-mediated transformation is the most studied biological method to T-DNA to plant cells, other organisms can also do this. Various *Rhizobium* species transfer DNA into plants, albeit at low frequencies compared with *Agrobacterium* (Hooykaas et al., 1977; Van Veen et al., 1988; Brothhaerts et al., 2005). *Ensifer adhaerens* has recently been shown to generate transgenic events in several species at frequencies similar to those produced by *Agrobacterium* (Wendt et al., 2012; Zuniga-Soto et al., 2015). Because *E. adhaerens* is not a plant pathogen, its use may also circumvent several regulatory hurdles. However, it is

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noteworthy that all microorganisms shown to transfer DNA to plants obligatorily use *Agrobacterium*-derived DNA transfer machinery. However, a *Rhizobium* species that encodes its own protein machinery capable of promoting DNA transfer and subsequent integration into the plant genome has recently been identified (Lacroix and Citovsky, 2016). Further refinement may yield transformation methods with properties distinct from those of *Agrobacterium*. In addition, further development of RNA viruses and geminiviruses may result in gene transfer protocols with superior genome editing properties.

### Synthetic Approaches to *Agrobacterium*-Mediated Plant Transformation

Insights into *Agrobacterium*-plant interactions, together with the emerging field of synthetic biology, may be applied to designing novel plant transformation technologies. If novel and unbiased synthetic methods were designed to transfer DNA from bacteria to the plant nucleus, several desirable features would be (1) high modularity facilitating adaptation to specific species and goals; (2) DNA transfer regardless of fragment size, plant species, or explant type; (3) transformation methods that do not trigger plant or bacterial defense responses; and (4) DNA integration into a specific locus bounded by well-defined borders. The design and fabrication of such a synthetic system could begin with native or disarmed *Agrobacterium* or *E. adhaerens* strains as natural platforms. The transformation components in the bacterium could be further streamlined to enable more precise engineering. Breakthroughs in synthetic biology make it possible to produce predictable functions from quantitatively characterized components and to refactor complex natural gene circuits into simpler designs that can then be optimized with desired parameters that are computationally selected (Smanski et al., 2014). These approaches applied to *Escherichia coli* plasmids and the nitrogen fixation gene cluster from *Klebsiella oxytoca*, among others, provide a roadmap to engineer a synthetic plant transformation platform (Temme et al., 2012; Smanski et al., 2014). For example, we should be able to refactor the *Agrobacterium* Ti-plasmid to have virulence and other functions that are temporally and quantitatively tuned for plant transformation, rather than for natural pathogenesis. To make the genetic components of a Ti-plasmid predictable, a detailed quantitative understanding of each component's transfer function is needed, not for pathogenesis, but for how these components contribute to plant transformation function. The *Agrobacterium* chromosomal DNA could be further "disarmed" to eliminate induction of unnecessary pathogenic responses and plant tissue necrosis. Theoretically, the upper limit to the amount of DNA that *Agrobacterium* can transfer is likely higher than currently practiced (Hamilton, 1997). Known limiters of DNA transfer size are the presence of cryptic or partial T-DNA border sequences within the T-DNA (Miranda et al., 1992). A redesign of the T-DNA border/transfer machinery might eliminate these problems. The use of transcription blocks and chromatin insulators is needed to avoid interference of adjacent genes and

genetic components. Transient induction of epigenetic components could allow chromatin reconfiguration and allow the T-DNA to function independently of the chromatin environment into which it initially integrates.

### "MODULAR" AGROBACTERIUM STRAINS AND BIOLISTIC DELIVERY SYSTEMS EASILY ASSEMBLED FOR USE IN THE PLANT BIOLOGY LABORATORY

Most plant transformation tools have been developed on an ad hoc basis and not to rational standards that would facilitate design and assembly of larger synthetic biological circuits from individual parts or from quantitatively defined transfer functions (Schaumberg et al., 2016). Designing "mix-and-match" modular components (Liu et al., 2013; Liu and Stewart, 2015) for delivery of biological molecules might be a more useful strategy for plant biology researchers. For example, "Phytobricks," similar to the Biobricks used in bacterial synthetic biology (Shetty et al., 2008), might be designed to carry swappable selectable markers, promoters, 3' untranslated regions, and insulators. A collection of synthetic constitutive, tissue-specific, and inducible promoters will be required to enable effective multigene metabolic engineering of plants (Liu and Stewart, 2016). Using Golden Gate (Engler et al., 2008), GoldenBraid (Sarrion-Perdigones et al., 2011), or other modular DNA assembly methods could facilitate building standardized parts for versatile transformation and genome editing. Due consideration must be given to the potential of "scars," such as the 3- to 4-bp fragments of DNA that are left by many of these techniques, that could alter gene expression and transfer functions.

### SUMMARY

Gene editing technologies have tremendous potential to enable increased understanding and manipulation of crop genomes. Transformation and regeneration of genome edited crops comprise a substantial current bottleneck that could be likened to a dial-up modem connection in the 1980s. Various technologies improved computer connectivity; a plethora of approaches will likely also be required to improve crop transformation. While floral dip transformation is an attractive solution inasmuch as it eliminates the need for tissue culture, it is only robustly reproducible in *Arabidopsis* and its relative *Camelina sativa* (Lu and Kang, 2008). Approaches to minimize tissue culture by manipulating cell and tissue development (Bouchabké-Coussa et al., 2013) might be the most robust strategy to deal with the tissue culture problem. An important research objective for plant biologists is to simplify crop transformation to such an extent that virtually any laboratory could do it. Improving the capacity and efficiency of plant transformation is a critical goal to maximize our implementation of crop genomics knowledge to feed the world.

### Supplemental Data

**Supplemental Table 1.** Plant Transformation Infrastructure: Public Transformation Facilities in the USA.



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## Advancing Crop Transformation in the Era of Genome Editing

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