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Recent advances in crop transformation technologies

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Agriculture is experiencing a technological inflection point in its history, while also facing unprecedented challenges posed by human population growth and global climate changes. Key advancements in precise genome editing and new methods for rapid generation of bioengineered crops promise to both revolutionize the speed and breadth of breeding programmes and increase our ability to feed and sustain human population growth. Although genome editing enables targeted and specific modifications of DNA sequences, several existing barriers prevent the widespread adoption of editing technologies for basic and applied research in established and emerging crop species. Inefficient methods for the transformation and regeneration of recalcitrant species and the genotype dependency of the transformation process remain major hurdles. These limitations are frequent in monocotyledonous crops, which alone provide most of the calories consumed by human populations. Somatic embryogenesis and de novo induction of meristems — pluripotent groups of stem cells responsible for plant developmental plasticity — are essential strategies to quickly generate transformed plants. Here we review recent discoveries that are rapidly advancing nuclear transformation technologies and promise to overcome the obstacles that have so far impeded the widespread adoption of genome editing in crop species.

The efficient production of transgenic plants relies on two key steps: transformation (the transfer and expression of transgenes into host cells) and regeneration (the ability to form a fertile plant from a transformed cell). For many species, transformation and regeneration are the bottlenecks to obtaining transgenic plants. Traditionally, callus induction from tissue explants has been the main avenue to produce transformed plants, either by providing undifferentiated cells for direct transformation or as a way to regenerate full plants from a few transformed cells (Fig. 1). However, callus formation is a lengthy procedure known to introduce genomic and epigenomic changes, often with unintended consequences[1,2]. Recent progress in both transformation and regeneration promises to quickly advance our ability to manipulate crop genomes. This includes the use of different morphogenic factors, genes that are involved in somatic embryogenesis or meristem development, to trigger reprogramming and pluripotency of a subset of somatic cells to eventually produce transformed plants[3].

Methods for plant transformation

The efficient delivery of gene-modification components into plant cells is a crucial first step of plant transformation and genome editing. Two major gene-delivery methodologies have been established in higher plants since the 1980s: direct gene transfer and *Agrobacterium*-mediated transformation[4] (Fig. 1). Direct gene transfer methods are good strategies to overcome possible competency barriers to transformation, and traditionally, microprojectile bombardment (also known as biolistics) has been used to facilitate the delivery of

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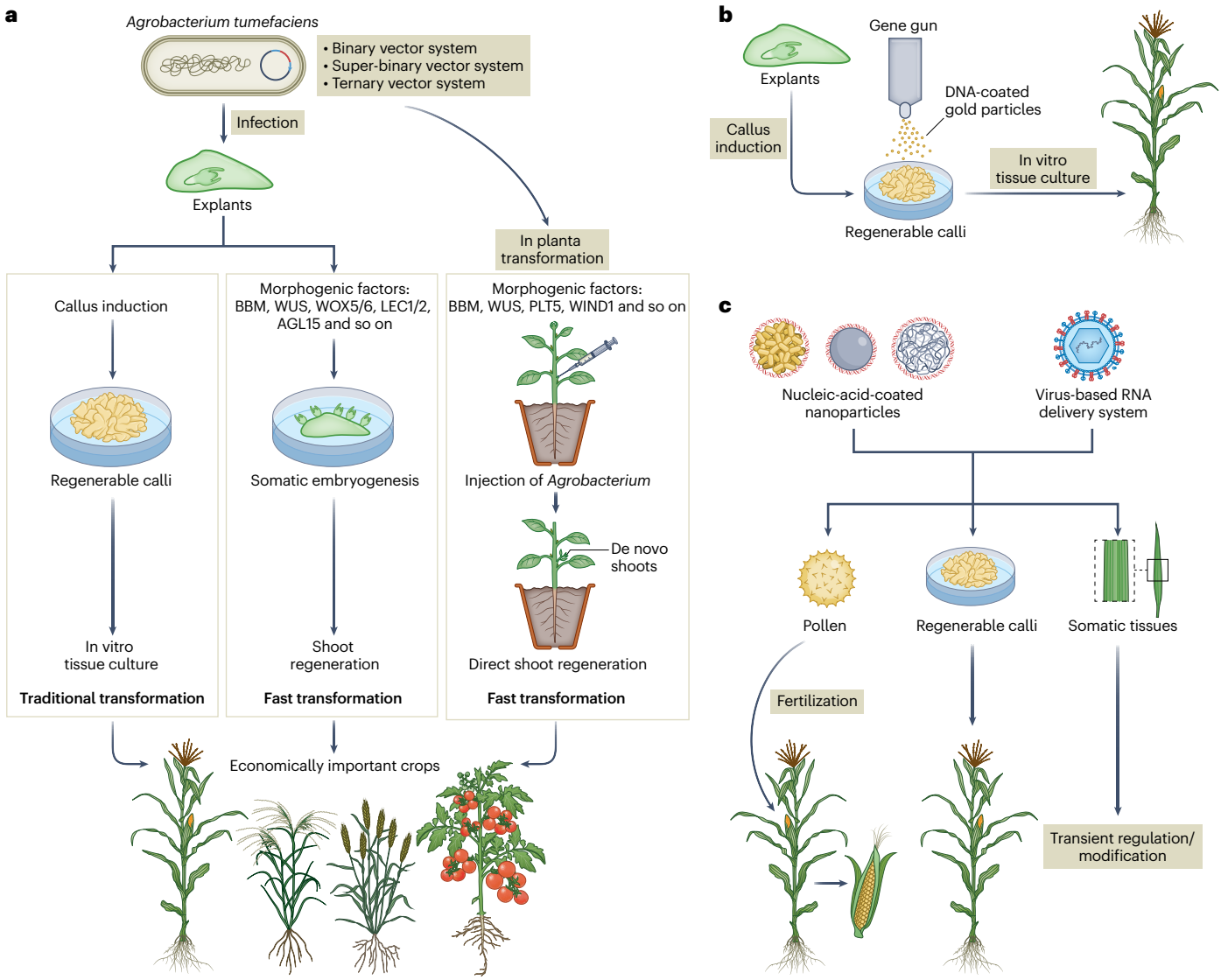

Fig. 1 | Plant transformation and regeneration strategies. a, *Agrobacterium*-mediated transformation strategies include callus induction followed by organogenesis (traditional transformation), somatic embryogenesis and de novo shoot regeneration in planta promoted by morphogenic genes (fast transformation). **b**, Particle bombardment for nuclear plant transformation. **c**, Various nanoparticles and virus-based RNA delivery systems for plant transformation. Created with BioRender.com.

transgenes in many species. Plants obtained from particle bombardments can contain multiple integration events and random rearrangements of the integrated copies with unpredictable effects, complicating the downstream analysis of the transgenic plants generated[5]. However, these phenomena are not unique to biolistic methods; they are sometimes observed in *Agrobacterium*-mediated transformations as well[6–10]. Recently, biolistic delivery has been used to directly provide in vitro transcripts or ribonucleoprotein complexes of CRISPR–Cas9 to regenerable plant tissues of a wider range of genotypes and species, obviating genome integration effects[11–14].

Agrobacterium-mediated transformation is a cost-effective, efficient gene-delivery system, capable of transferring large DNA fragments into plant chromosomes[15], and remains the top choice for plant transformation. However, only a small range of plant host genotypes are competent for *Agrobacterium* infection, and in numerous monocot crops, ineffective *Agrobacterium* infection hinders its widespread application. To improve transformation efficiency, substantial efforts have been made to optimize virulence (*vir*) gene expression of *Agrobacterium* strains. Progress in our understanding of the mechanisms used by *Agrobacterium* to modulate plant host defence responses for effective infection and of the role of phytohormones in the early stages of plant–*Agrobacterium* interaction is summarized in a recent review[16]. The process of T-DNA transfer starts with a two-component system containing the hybrid histidine kinase VirA and the response regulator VirG. The VirA protein is activated by phenolic compounds (such as acetosyringone, a common ingredient in transformation media) and triggers the phosphorylation of VirG, which induces the expression of other *vir* genes in the Tumor-inducing (Ti) plasmid[16,17]. The *vir* gene expression can be repressed by phytohormones from the host side at the early stage of plant–*Agrobacterium* interaction. In particular, salicylic acid (SA) prevents the induction of *vir* genes by attenuating the kinase function of the VirA protein[16,18]. Consequently, plants that overproduce SA are recalcitrant to *Agrobacterium* infections, whereas those with defective SA biosynthesis are easier to transform[18,19]. The negative effect of SA on the VirA/G two-component regulatory system can be alleviated by the addition of acetosyringone[18]. Different *Agrobacterium* strains containing binary, super-binary and recently emerged ternary vectors have been developed to fully exploit the

power of the *vir* genes[16]. Specifically, improved ternary vector systems introduce a third helper plasmid containing extra *vir* genes and have been shown to enhance transformation efficiency in recalcitrant maize lines[20,21]. These strains, combined with a fast-transformation protocol, increased transformation efficiency in the commonly used maize inbred line B104 (ref. [22]). Recently, engineered *Agrobacterium* strains expressing a type III secretion system to deliver *Pseudomonas* effectors that repress host defence responses resulted in increased transformation efficiency in wheat, alfalfa and switchgrass[23].

Nanoparticle[24] and virus-based RNA delivery systems can bypass tissue culture and are attracting increasing attention for efficient genome editing. Several nanomaterials, such as silica, metal, polymeric and magnetic nanoparticles as well as carbon nanotubes, have been investigated for their potential role in gene delivery for plant transformation. Although recently questioned[25], a novel transformation methodology called pollen magnetofection was reported to allow the stable integration of transgenes in the nuclear genome of several dicot species[26]. In this technology, recombinant DNA is attached on the positively charged surface of magnetic nanoparticles that can penetrate pollen grains in a magnetic field; subsequent fertilizations are conducted to complete the transformation process[26,27].

Virus-based RNA delivery systems have the additional advantage of bypassing transgene integration, thereby avoiding possible disruptive effects on gene function. RNA-virus-based vector systems have been developed to express guide RNA arrays for multiplex genome editing in several species. In tobacco, a rhabdoviral system was developed to deliver all CRISPR–Cas9 components during infections; however, a lack of editing in the germline required a tissue-culture step to obtain edited progenies[28]. An alternative approach was pursued in both wheat and maize in which the viral system relied on infections of previously obtained Cas9-positive plants, and these systems generated edited plants with high efficiency in both species[29,30]. These virus-based systems are still limited by host range and physical constraints but are nonetheless very promising approaches for genome editing of crop species and for breeding purposes.

Molecular mechanisms of plant regeneration

Although the remarkable capacity to regenerate organs and entirely new individuals is considered a signature feature of plants[31], many crop species are incapable of naturally regenerating whole plantlets upon loss or injury of body parts[32]. Nevertheless, in vitro cultivation of various types of explants on enriched media supplemented with specific phytohormones enables many plant species to regenerate plantlets (Fig. 1). The regeneration process typically involves a callus-formation step followed by organ differentiation, direct embryogenesis from somatic tissues (also known as somatic embryogenesis) or de novo formation of meristems. The capacity to regenerate meristems or whole plantlets from non-zygotic cells is the fundamental basis for agricultural and horticultural biotechnology, and it allows both clonal propagation and the production of stable transgenic material for breeding. Regardless of the substantial technological progress that has been made over the past four decades, there are still many economically important crops that are recalcitrant to in vitro regeneration from somatic tissue. Understanding the molecular mechanisms governing plant regeneration is therefore particularly critical for accelerating plant biotechnology.

Auxin and cytokinin are key hormones for shoot and root apical meristem formation and de novo organogenesis[31,33,34]. The fate of explants under in vitro cultivation follows the golden hormonal regeneration rule that has been successfully applied to in vitro propagation of many plant species: a high cytokinin-to-auxin ratio stimulates shoot formation, while a reverse ratio promotes root formation. Auxin is a versatile regulator and plays an essential role in embryogenesis by establishing both the shoot apical and root apical meristems[35]. In particular, auxin biosynthetic genes, such as *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS* (*TAA1*), *TAA-RELATED 1* and *2* (*TAR1,2*), and several flavin monooxygenase-like *YUCCA* (*YUC*) genes, regulate early embryogenesis[36,37]. In practice, auxin (mostly the synthetic auxin-like hormone 2,4-dichlorophenoxyacetic acid (2,4-D)) typically serves as a major inducer for somatic embryogenesis from explants of different origin. During 2,4-D-induced somatic embryogenesis in *Arabidopsis*, genome-wide changes in chromatin accessibility, enhanced expression of auxin pathway genes and changes in key developmental regulators are observed in a hierarchical cascade upstream of early embryonic patterning genes[38–40] (Fig. 2). Increasing evidence points to the importance of locally elevated endogenous auxin biosynthesis, mainly indole-3-acetic acid, as key to somatic embryogenesis[41–43]. Although the specific mechanism by which auxin biosynthesis promotes somatic embryogenesis has not been fully resolved, auxin biosynthetic genes are direct targets of the B3 transcription factor LEAFY COTYLEDON 2 (LEC2), whose inducible overexpression is sufficient to promote *Arabidopsis* somatic embryogenesis independent of tissue competence, which is normally restricted to immature zygotic embryos[40,44]. A similar stimulating effect on somatic embryogenesis was originally reported with the overexpression of *LEC1*, another transcriptional regulator[45].

Cytokinins are adenine-derived molecules that control meristem size by directly regulating the expression levels of the homeobox transcription factor WUSCHEL (WUS), which plays a central role in meristem establishment and maintenance in most species. Extensive studies in *Arabidopsis* have shown that cytokinin biosynthesis and signalling provide positional cues for WUS patterning in meristems via both positive and negative feedback loops[31]. During in vitro shoot regeneration experiments in *Arabidopsis*, the treatment of explants with high concentrations of cytokinin removed the repressive H3K27me3 histone modification at the *WUS* locus and allowed transcriptional activators of the cytokinin signal transduction pathway to promote *WUS* transcription for de novo formation of shoot meristems[46]. ATAC-seq and ChIP-seq profiling during callus induction and shoot regeneration in *Arabidopsis* provide a molecular timeline where auxin and cytokinin contribute to the dynamic regulation of chromatin state and the accessibility of key transcription factors involved in both promoting pluripotency acquisition during callus induction (in a high-auxin/cytokinin environment) and shoot fate determination during regeneration (in low-auxin/cytokinin conditions)[47].

Wounding is a primary signal not only for naturally occurring organ repair but also for plantlet regeneration. In vitro shoot regeneration experiments from root explants of *Arabidopsis* have shown that this process typically involves transcriptional activation of multiple developmental regulators, including the AP2/ERF transcription factors ENHANCER OF SHOOT REGENERATION 1 (ESR1) and WOUND INDUCED DEDIFFERENTIATION 1 to 4 (WIND1 to 4). WIND1 was found to activate the expression of *ESR1* and promote shoot regeneration[48,49]. Another AP2/ERF transcription factor, ETHYLENE RESPONSE FACTOR115 (ERF115), forms a heterodimeric complex with PHYTOCHROME A SIGNAL TRANSDUCTION1 (PAT1) to re-establish a stem cell niche upon root tip excision, and its high regenerative potential is correlated with the activation of WIND1, one of its putative targets[50]. Moreover, transcriptomic analysis on *Arabidopsis* hypocotyl excisions revealed that three additional AP2/ERF transcription factors, PLETHORA 3 (PLT3), PLT5 and PLT7, are wound-induced and promote callus formation[51]. *PLT* genes lead to the induction of *YUC1* and auxin-dependent activation of cell cycle regulators during callus formation from *Arabidopsis* protoplasts, providing a mechanistic model of pluripotency acquisition in differentiated cells[52]. These three factors are key regulators of lateral root development, and the transient induction of *PLT5* or *PLT7* from various *Arabidopsis* explants (or *PLT5* in snapdragon and tomato) is sufficient to trigger de novo shoot formation in a hormone-independent manner[53–55].

Wounding is also an important signal for plant glutamate-receptor-like proteins (GLRs), which have a well-documented role in defence responses. The role of glutamate receptors in regeneration

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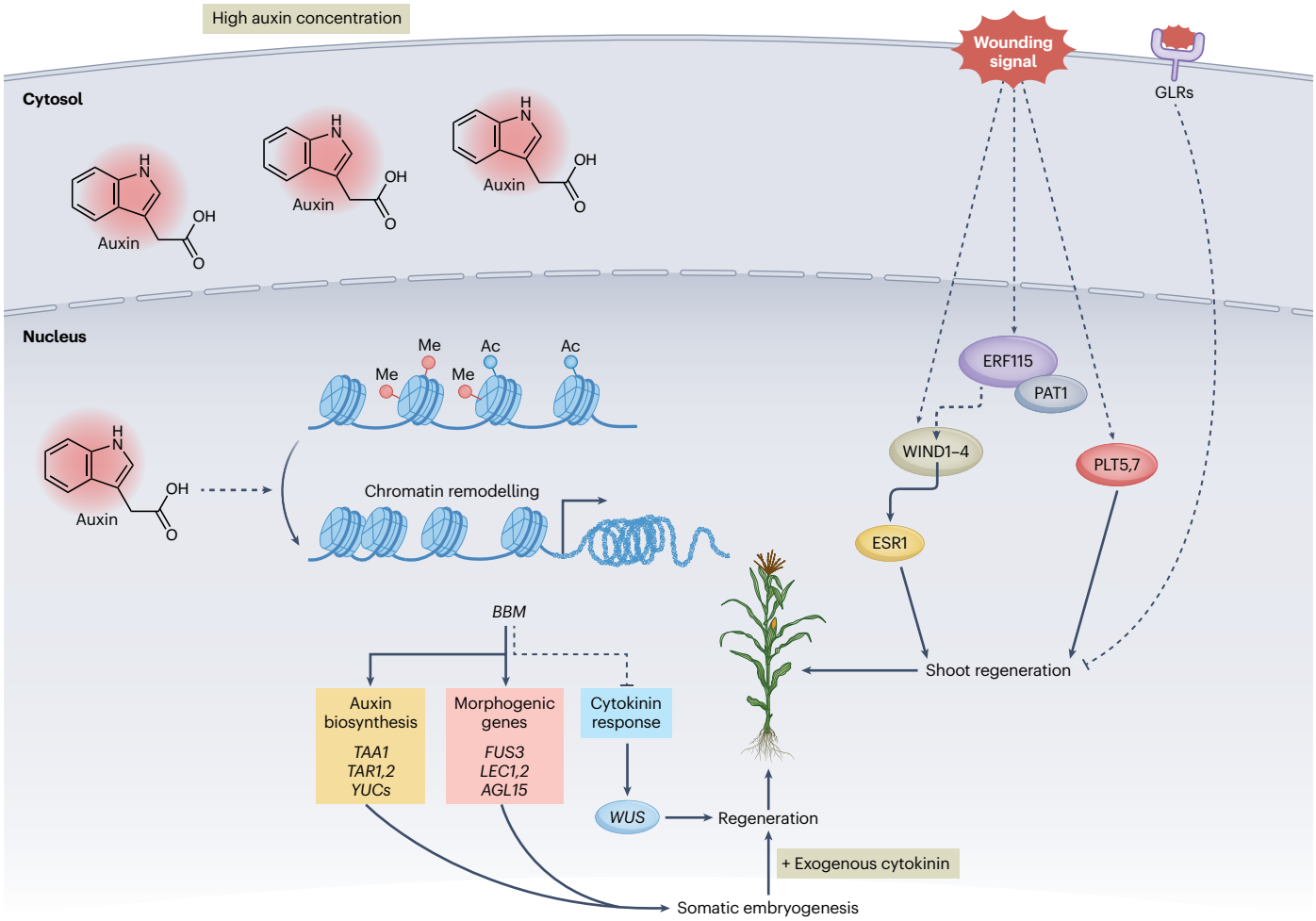

Fig. 2 | Plant regeneration pathways. Known molecular components of plant regeneration pathways. Solid lines indicate well-established relationships, while dashed lines indicate relationships where key molecular details are unknown; arrowhead ends indicate positive regulation and perpendicular ends indicate negative regulation. Created with BioRender.com.

is mediated by SA signalling, and mutants in the SA receptor NPR1 are hyper-regenerative and partially resistant to GLR perturbations, suggesting that a higher efficiency of *Agrobacterium*-mediated transformation could be obtained by attenuating the SA response. Indeed, genetic and pharmacological inhibition of GLR activity increased the regeneration efficiency of multiple organ repair systems in *Arabidopsis* and the callus induction rate of the recalcitrant maize inbred line B73. These results suggest that the regulation of the trade-off between defence and regeneration can be harnessed to improve regeneration for agricultural purposes[56] (Fig. 2).

Somatic embryogenesis shares several developmental steps with zygotic embryogenesis, and key trigger genes of embryogenesis initiation can be manipulated to induce somatic embryogenesis for whole-plant regeneration. The AP2/ERF transcription factor BABY BOOM (BBM, also known as PLT4 in *Arabidopsis*) promotes somatic embryogenesis[57–59] and directly regulates several auxin biosynthesis genes, including *YUC* genes in both monocot and dicot species. Pharmacological and genetic inactivation of endogenous YUC activity drastically reduced somatic embryogenesis via BBM induction in both rice and *Arabidopsis*, indicating that endogenous indole-3-acetic acid levels cannot be substituted by the 2,4-D present in the medium[41,60]. A recent study suggests that this may be due to differential effects of exogenous and endogenous auxin on cell cycle progression[52]. During somatic embryogenesis, YUC-dependent auxin biosynthesis does not appear to be required for re-establishing pluripotent cells but remains essential for embryo identity and growth[41]. BBM also functions directly upstream of the above-mentioned transcription factor LEC2 and is in turn upregulated by LEC2 in a reinforcing loop during somatic embryogenesis[40,61]. In rice, the *BBM1* gene can promote parthenogenesis, the formation of embryos without fertilization, when ectopically expressed in unfertilized egg cells[58]. Similarly, the recently isolated *PARTHENOGENESIS* (*PAR*) gene of apomictic dandelion, encoding a K2-2 zinc finger and EAR-domain containing protein, can trigger embryogenesis in unfertilized egg cells, and its ectopic expression in unfertilized egg cells of lettuce is sufficient to induce the formation of haploid embryos[62]. While yet to be determined, it is possible that *PAR* and other parthenogenic genes could be used to promote somatic embryogenesis for whole-plant regeneration in certain species.

The roles of other genes, such as *FUSCA 3*, *LEC1* and *2*, and *AGAMOUS-LIKE 15* (*AGL15*), in promoting somatic embryogenesis have been well described in *Arabidopsis* and other species[3,63] (Table 1), and the expression of these morphogenic genes is believed to be regulated by BBM[61,64]. However, whether these genes can be applied to stimulate regeneration in a broad range of species has yet to be determined.

Morphogenic factors for crop transformation

A significant breakthrough technology in plant transformation, especially for monocot species and species recalcitrant to transformation, has been the exploitation of specific morphogenic factors to reprogram somatic cells into initiating embryogenesis. This has spurred a renewed interest in exploiting specific developmental regulators for crop transformation[3,63,65] (Table 1). These factors include BBM, WUS

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Table 1 | Successful examples of utilization of morphogenic genes for transformation of crop species

Individual genes	Gene name	Transformed and regenerated crop	Relevant notes	References
AGL15	Gm*AGL15*	Soybean	Promotes somatic embryogenesis.	94
WUS/WOX	Ta*WOX5*	Wheat, maize, triticale, rice, barley	With ternary vector system.	70,77
	At*WUS*	Coffee	Promotes somatic embryogenesis.	95
	Zm*WUS2*	Sorghum, maize	With ternary vector system.	68,96
	Brr*WUSa*	Turnip	Callus step required.	97
PLT	At*PLT5*	Tomato, snapdragon	Organogenesis at wound site.	55
KN1/STM	Zm*KN1*	*Citrus*	Promotes organogenesis.	98
	Bn*STM* Bo*STM* Br*STM*	Rapeseed	Promotes microspore-derived embryogenesis.	99
BBM	Bn*BBM*	Sweet pepper	Promotes somatic embryogenesis.	100
	Bc*BBM*	Chinese white poplar	Promotes somatic embryogenesis.	101
GRF	At*GRF5*, Ha*GRF5*, Gm*GRF5*, Bn*GRF5*, Bv*GRF5*, Zm*GRF5*, Zm*GRF5-LIKE1*	Sugar beet, soybean, maize	Callus step required or promotes organogenesis from explants.	84
Gene combinations				
WUS BBM	*ZmWUS2* Zm*BBM*	Maize, sorghum, rice, sugarcane, salvia, wheat	Promotes somatic embryogenesis. Use of specific maize promoters or excision required to avoid developmental defects. Uses ternary or super-binary vector.	59,67,102
GRF–GIF	Ta*GRF4–GIF1*	Wheat, rice, triticale, *Citrus*, watermelon, hemp	Callus step required.	71,83,103
GRF–GIF BBM	Ta*GRF4–GIF1* Zm*BBM*	Maize	Promotes somatic embryogenesis.	89
WUS IPT WUS STM	Zm*WUS2* *IPT* At*STM*	Tomato, potato, grape	Promotes de novo meristem initiation at wound sites. Many edited plants show developmental defects and are sterile.	66

At, *Arabidopsis thaliana*; Ta, *Triticum aestivum* (bread wheat); Zm, *Zea mays* (corn); Tc, *Theobroma cacao* (cacao); Bn, *Brassica napus* (rapeseed); Bo, *Brassica oleracea* (cabbage); Br, *Brassica rapa* (mustard); Brr, *Brassica rapa* var. *rapa* (turnip); Bc, *Brassica campestris* (mustard); Gm, *Glycine max* (soybean); Gh, *Gossypium hirsutum* (cotton); Bv, *Beta vulgaris* (sugar beet); Ha, *Helianthus annus* (sunflower).

and WUSCHEL-RELATED HOMEOBOX (WOX), which are key regulators of embryogenesis initiation and meristematic stem cell fate, as summarized in the previous section. Individual or combined expression of these regulators in somatic cells is often sufficient to trigger whole-plant regeneration under in vitro cultivation or de novo shoot formation on soil-grown plants[66]. While detailed reviews on the use of morphogenic genes have been published[3,63,65], here we focus on the most recent advances using these strategies specifically for the transformation of crop species.

Ectopic expression of the maize *BBM* and *WUS2* genes (a co-orthologue of *Arabidopsis WUS*) increased transformation efficiency in several crops, including maize, sorghum, indica rice, sugarcane and genotypes recalcitrant to biolistic and *Agrobacterium*-mediated transformation[59]. Additionally, combinatorial expression of *WUS2* together with different developmental regulators (including the cytokinin biosynthesis *IPT* and the meristematic *STM* genes) at the wound sites of soil-grown plants induced de novo meristem formation and enabled gene editing, bypassing the tissue-culture step in tomato, potato and grape[66].

However, constitutive expression of morphogenic genes is usually not well tolerated, often resulting in infertile plants or plants with undesired pleiotropic phenotypes. While these undesired effects can be segregated away for genome-editing approaches, alternative strategies to express these factors transiently (only during a specific stage) or to entirely remove them post-infection have been pursued[3,59,67]. In a recent strategy to overcome this issue, the non-cell autonomous function of ZmWUS2 was exploited to produce transformed plants without the Zm*WUS2* expression cassette, a phenomenon designated as altruistic transformation. In this system, two independent strains of *Agrobacterium*, one containing the selectable marker and the other the cassette expressing Zm*WUS2*, were successfully used in different ratios for maize embryo infections[68]. Similarly, a recently developed CRISPR-based approach to simultaneously induce the expression of endogenous morphogenic genes and promote editing increased the recovery of transformed and edited plants without developmental defects in poplar and rice, offering a new tool for accelerating genome engineering[69].

Recent studies have shown that constitutive expression of either WOX5 or a GROWTH REGULATING FACTOR4 (GRF4) and GRF-INTERACTING FACTOR1 (GIF1) chimaeric protein enhances transformation efficiency in wheat as well as several other monocot species[70,71]. While both systems still require a callus-induction stage, they were shown to expand the range of genotypes that can be regenerated, a major limitation to the widespread and fast adoption of transformation technologies in crops. WOX5 belongs to the same homeobox family of WUS and plays a key role in root stem cell maintenance, and its overexpression induces shoot regeneration in *Arabidopsis* calli, possibly by promoting *TAA1* auxin biosynthesis and interfering with cytokinin signalling[72–76]. The overexpression of a wheat *WOX5* gene significantly enhanced the transformation efficiency in recalcitrant genetic backgrounds in wheat, barley and maize, seemingly without detrimental effects on overall development, making it an attractive system for monocot transformation[70]. However, in maize, *WOX5* was tested in combination with a ternary

vector system; therefore, whether *WOX5* itself is responsible for the reported efficiency has yet to be determined[77].

GRFs are highly conserved, plant-specific transcription factors that promote growth and boost organ size when overexpressed[78–80]. GRFs form protein complexes with GIF cofactors that in turn interact with SWI/SNF chromatin remodelling factors[81,82]. By forcing physical proximity of both proteins in the GRF–GIF chimaeric protein, the system proved efficient for transformation by increasing regeneration not only in durum and bread wheat but also in rice, triticale and the dicot crops *Citrus*, watermelon and hemp[71,83]. Furthermore, the GRF–GIF chimaera allowed wheat shoot regeneration in media lacking cytokinin, allowing marker-free selection of transgenic plants and suggesting a potential role of the GRF–GIF complex in the regulation of endogenous cytokinins[71]. The use of individual GRF factors has been reported to improve transformation efficiency in sugar beet, canola, sunflower and maize without affecting the development or fertility of transgenic plants[84]. While the molecular mechanisms of how GRF–GIF complexes promote regeneration are currently not fully known, the direct downstream targets of rice OsGRF6 included auxin biosynthesis and signalling genes, suggesting that GRF–GIF complexes promote regeneration by elevating auxin biosynthesis and auxin signalling in transformed cells[85]. In addition, a regulatory loop between *GRF* and *PLT* genes was reported to establish the boundary between the stem cell niche and the transit-amplifying region in *Arabidopsis* root meristems, suggesting that GRF–GIF complexes could influence PLT activity[79].

Concluding remarks and future perspectives

Despite the recent breakthroughs using morphogenic factors, challenges still remain in developing fast, efficient and reliable transformation systems that can be quickly adopted by the public sector, particularly for monocot species. Given that some of these morphogenic factors function in hierarchical order during embryogenesis and meristem formation[40,47], their combination may promote somatic embryogenesis in an additive manner (for example, BBM WUS2)[59,67,68,86–88] (Table 1), even when the molecular mechanisms driving these synergistic interactions are not entirely known[59,66,67]. Indeed, preliminary data suggest that a higher efficiency of maize embryo transformation is achievable using a combination of the wheat GRF–GIF chimaera and the maize BBM transcriptional regulator with standard *Agrobacterium* binary vector systems[89] (Table 1). The identification of additional genes involved in the regeneration process may foster additional combinations that could prove optimal for the transformation of certain recalcitrant crop species or genotypes. The selection of optimal combinations would benefit from additional insights into the regeneration process, which should be obtainable from single-cell chromatin accessibility and expression data of specific species, genotypes and organs[76]. These studies may also aid the identification of molecular signatures of precursor cells during somatic embryogenesis and provide new targets for biotechnological approaches to transformation.

Despite recent progress, the tissue and genotype dependency of the transformation process still represent major bottlenecks to the widespread use of transgenic and genome-editing technologies in many economically important crop species. Some of the technologies discussed here have been tested with success in different tissues, including embryos excised from dry seeds and leaf segments from seedlings[59]. An ideal system should use reliable and easily accessible sources of tissue (for example, leaves, coleoptiles or root tips) or even protoplasts[90] for transformations, and specific morphogenic triggers to overcome their intrinsic lack of competency for somatic embryogenesis and regeneration[40]. In species such as maize and wheat, this would facilitate the widespread adoption of transformation technologies in academic labs, whereas in species clonally propagated or with embryos too small to handle, it may represent the only viable strategy.

To accelerate crop transformation and breeding, it is also imperative to develop new DNA delivery methods with reduced genotype dependency, which may involve the use of new bacterial strains with high efficiency of infection[91] or the adoption of specific nanomaterials for transformation. Promising advances with ternary vector systems for *Agrobacterium*-mediated transformation[20,21] may more broadly tackle the transformation of recalcitrant backgrounds of different crops and may be adaptable to different transformation systems. The identification of new genes and pathways that confer genotype dependency of transformation may also contribute to solving this issue, as shown in maize, where knocking out *SAUR15* (an early auxin-responsive gene) significantly increased regeneration efficiency[43]. Pursuing a variety of these approaches will probably be key to increase the adoption of transformation technologies in recalcitrant crops and genetic backgrounds, including wild progenitors and diverse germplasms[92,93], for both basic and applied research.

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Author contributions

Z.C., J.M.D., J.D. and A.G. conceived the manuscript, contributed to writing and editing, and approved the manuscript.

Competing interests

J.M.D. is co-inventor in patent no. US2017/0362601A1, which describes the use of chimaeric GRF–GIF proteins with enhanced effects on plant growth (Universidad Nacional de Rosario Consejo Nacional de Investigaciones Científicas y Técnicas). J.D. and J.M.D. are co-inventors in UC Davis patent application no. WO2021007284A2, which describes the use of GRF–GIF chimaeras to enhance regeneration efficiency in plants.

Additional information

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