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Bypassing Tissue Culture: How In Planta Transformation is Rewriting Plant Biotechnology

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For decades, plant genetic engineering has leaned heavily on sterile petri dishes, complex hormone cocktails, and the delicate art of tissue culture. While classical tissue culture-based protocols powered the first wave of agricultural biotechnology, they carry a major bottleneck: recalcitrance. Many elite commercial crops flatly refuse to regenerate from dedifferentiated callus cells, turning gene editing and transgenesis into grueling, labor-intensive endeavors plagued by somaclonal mutations and genotype dependency. *In planta* transformation sidesteps this entire in vitro bottleneck. By delivering genetic payloads directly to intact plant tissues—saving time, lab infrastructure, and reagents—it enables plants to grow in natural conditions while carrying heritable modifications straight to their progeny.

Techniques at a Glance

- **Floral Dip:** Dipping intact inflorescences into an Agrobacterium suspension to transform female gametophytes directly; routine and universally established in Arabidopsis.
- **Vacuum Infiltration:** Applying negative pressure to force bacterial suspensions deep into stomata and internal leaf or stem air spaces.
- **Embryo Transformation:** Delivering vectors directly into the shoot apical meristems (SAM) of imbibed or mature seed embryos, bypassing callus induction entirely.
- **Seedling & Meristem Inoculation:** Transforming young intact seedlings through needle piercing, stem severing, or direct micro-injection.

Biological Hurdles of Direct Transformation

Despite its elegance, direct in planta delivery presents several technical challenges that researchers must engineer around:

- **Subdued Transformation Frequency:** Direct uptake in non-regenerative, intact tissues is frequently lower than in optimized, highly proliferative callus culture lines.
- **Chimerism:** Transformed meristematic cells often divide alongside wild-type cells, producing genetic mosaics where transformed sectors may fail to enter the germline.
- **Absence of Early In Vitro Filters:** Without early antibiotic or herbicide selection plates, researchers face heavier screening overhead in subsequent progeny generations.
- **Morphological, Architectural, and Cuticular Barriers:** Thick cell walls, waxy cuticles, and structural vascular defenses can impede both bacterial vectors and biolistic microprojectiles.

Stable In Planta Breakthroughs

Optimized delivery platforms have systematically tackled these barriers across diverse species, demonstrating verified germline transmission and Mendelian inheritance:

Species / Cultivar	Delivery Platform	Target Tissue & Mechanics	Integrated Genes & Promoters	Validated Efficiency & Stability	Reference
Wheat (*Triticum aestivum*) cvs. Fielder & Haruyokoi	In Planta Biolistics (iPB)	Exposed embryonic SAM; 0.6 µm gold particles delivered at 1350 psi into the L2 layer	GFP reporter construct	62% T0 germline transmission; Mendelian T1 inheritance; zero somaclonal artifacts	Hamada et al. (2017)
Thale Cress (*Arabidopsis thaliana*)	Meristem Severing	Shoot severing at rosette base + A. tumefaciens stump inoculation	pBI121 (uidA / GUSint + nptII)	5.5% shoots formed transgenic progeny; verified T2–T4 Mendelian segregation	Chang et al. (1994)
Tomato (*Solanum lycopersicum*)	Needle-Piercing & Incubation	3-day-old embryonic SAM pierced + 20-minute bacterial bath	Lip9 :: DREB1A + hpt	Stable integration validated by RT-PCR & Southern blot; T2 cold tolerance confirmed	Shah et al. (2015)
Sweet Potato, Potato, Bayhops	RAPID Platform	Direct micro-injection into active vegetative axils/meristems	Reporter cassettes & functional genes	Direct generation of non-chimeric vegetative propagules; complete sterile culture bypass	Mei et al. (2024)
Sesame (*Sesamum indicum*) cv. Sohag 1	Non-Invasive Agro-Inoculation	Intact vegetative seedlings inoculated in situ	bar gene + nptII backbone marker	65.6% T0 PCR-positive; confirmed via T1 bar RT-PCR, backbone exclusion (nptII-negative), and Basta® tolerance	Sultan & Tawfik (2023)

The Horizon

Eliminating tissue culture recalcitrance removes one of the steepest bottlenecks in modern agricultural genomics. As highlighted by Chang et al. (1994), Hamada et al. (2017), and Shah et al. (2015), targeting embryonic or meristematic zones can reliably secure germline transmission without somaclonal artifacts. Furthermore, recent advancements such as the RAPID platform by Mei et al. (2024) and non-invasive seedling inoculation by Sultan & Tawfik (2023) prove that in planta methods are viable across both seed- and vegetatively

propagated crops. Coupled with CRISPR/Cas ribonucleoprotein (RNP) delivery and nanoparticle-mediated gene shuttles, in planta systems provide an accelerated, DNA-free route to produce genome-edited, climate-resilient crops in significantly compressed breeding cycles—taking modern biotechnology straight from the seed to the open field.

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