

Unit 4: Biotransformation and Transgenesis

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4.1 Biotransformation

Biotransformation is the use of living cells, here cultured plant cells, to carry out a specific chemical conversion on a supplied substrate, the cell acting as a selective enzymatic reagent. Plant cells perform the reaction types of a well stocked enzymatic bench: hydroxylation, glycosylation, oxidation and reduction, hydrolysis, methylation and related modifications, often with a regio and stereo selectivity that ordinary chemistry reaches only with difficulty. The pharmaceutical logic should be stated as the syllabus frames it, conversion of a lower value or inactive precursor into a higher value product in one biological step. The classical textbook illustration is the conversion work of *Digitalis* cell cultures on supplied cardenolide substrates, modifying the molecule at positions chemistry handles poorly, taught in every standard text as the model of plant cell biotransformation. Biotransformation differs from production in Section 3.5 in one clean line: in production the cell builds the product from simple nutrients through its whole pathway, while in biotransformation the scientist supplies a late, complex substrate and the cell performs one or a few steps. Its practical conditions follow: the substrate must reach the cells and not poison them, immobilised cells of Unit 3 make an especially convenient reusable catalyst bed for the purpose, product recovery is from cells or medium according to where the product accumulates, and the economics work where the step is hard chemically, the substrate is cheap, or the product value is high. Its limits are equally plain: conversions can be slow, substrate loading is modest, and side reactions by other cell enzymes can complicate purification.

4.2 Bioreactors for pilot and large scale cultures of plant cells

A bioreactor is the vessel system in which a culture is grown at scale under monitored, controlled conditions, and plant cell bioreactors are adaptations of microbial fermentation hardware to a more difficult tenant. Plant cells are large, aggregate readily, are damaged by shear, grow slowly, and demand oxygen without tolerating the violent aeration microbes accept; reactor choice is therefore dominated by gentle mixing and aeration. The main types should be known with their working character. The stirred tank reactor, borrowed from microbial work, mixes with an impeller and sparges air; it is versatile and well instrumented but its shear is the highest of the family, managed by low speeds and adapted impellers. The airlift reactor has no impeller: rising air in a central draft tube circulates the liquid, giving low, even shear and good oxygen transfer for suspensions, and it is the type most associated with plant cell scale up. Bubble column reactors are the simplest aerated columns, gentle and cheap, with less control. Specialised forms serve organised cultures: temporary immersion and mist or trickle bed systems bathe shoots and hairy roots periodically or continuously in medium without drowning or shredding them, since a root mass or shoot cluster cannot be stirred like a suspension. Pilot scale work proves the culture in such a reactor at intermediate size, establishing the inoculum train, the feeding and harvest schedule, and the control of the standard variables, temperature, pH, dissolved oxygen and sterility, before large scale commits money and months to a slow growing culture. The types are compared in the diagram below.

Bioreactors for plant cells

STIRRED TANK	AIRLIFT AND COLUMN	FOR ORGANISED CULTURE
Impeller mixing, air sparged	Airlift: air drives circulation	Temporary immersion systems
Versatile, well instrumented	No impeller, low even shear	Mist and trickle beds
Highest shear of the family	Good oxygen transfer	For roots and shoot clusters
Low speed, adapted impellers	Bubble column: simplest form	No stirring of the tissue
Borrowed from microbial work	Classic plant cell scale up	Medium bathes, then drains

Gentle mixing and oxygen for slow, shear sensitive cells is the design rule

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Figure: Bioreactor types for plant cells. The design question is always the same: deliver oxygen and mixing to slow, shear sensitive cells without injuring them.

4.3 Retention of biosynthetic potential in cell culture

Retention of biosynthetic potential is the keeping, by a cultured line, of its ability to make the constituents it was selected to make, across subcultures and across scale up. The syllabus joins it to bioreactors deliberately, because the commonest industrial disappointment of the subject is a line that produced well in flasks and fades in the reactor or after months of transfer. The causes of loss collect the lessons of the whole course: somaclonal and epigenetic drift under culture conditions, Sections 2.5 and 3.4; selection pressure running the wrong way, since fast growing low producing cells outgrow slow high producers in every transfer, so routine subculture silently selects against production; loss of differentiation in lines whose pathway needs it; and stress or changed conditions at scale. The retention strategies answer cause by cause. Preserve master lines at an early, verified stage, by slow growth storage or cryopreservation where established, and restart working lines from the master rather than transferring indefinitely. Screen and reselect periodically, assaying the line, not assuming it. Keep selection pressure honest by subculturing from producing material and by monitoring constituent content as a routine culture parameter beside growth. Favour stable systems, hairy roots and organised cultures, for pathways prone to fade. And treat the passage from flask to pilot reactor as a retention test in itself, Section 4.2, verifying production at each scale before proceeding. Retention is therefore a management discipline laid over the biology: the genome holds the potential, and the culture system either keeps the conditions under which it is expressed or loses it by degrees.

4.4 Transgenic plants

A transgenic plant is a plant whose genome carries, stably integrated, DNA introduced by the gene transfer methods of Section 2.11, inherited by its progeny and expressed as a new or altered trait; the whole enterprise is transgenesis. The construction logic should be known in outline: the gene of interest is assembled in a vector with a promoter to drive its expression in the right tissue and at the right strength, a terminator, and a selectable marker; transfer follows by the *Agrobacterium* or direct routes; transformed cells are selected and regenerated; and the plants are verified and characterised, presence and structure of the insert, its expression, and the trait, before any use. In medicinal plant work the trait targets follow the syllabus objectives: higher yield of a phytopharmaceutical through added or strengthened pathway genes; a cleaner profile through silencing of a gene making an unwanted constituent or diverting a shared precursor; resistance to disease, pests or stress that stabilises production in the field; and production of valuable proteins and other products in plant systems. Transgenic hairy roots, carrying both the root inducing genes

and added pathway genes, are a laboratory production form of the same idea. The cautions complete a balanced answer: expression varies with insertion site and copy number and can be silenced in later generations, containment and regulatory assessment govern any release, and a transgenic line, like a culture line, must be verified continuously. The methods compared visually below are the delivery half of transgenesis; the verification half is the next two sections.

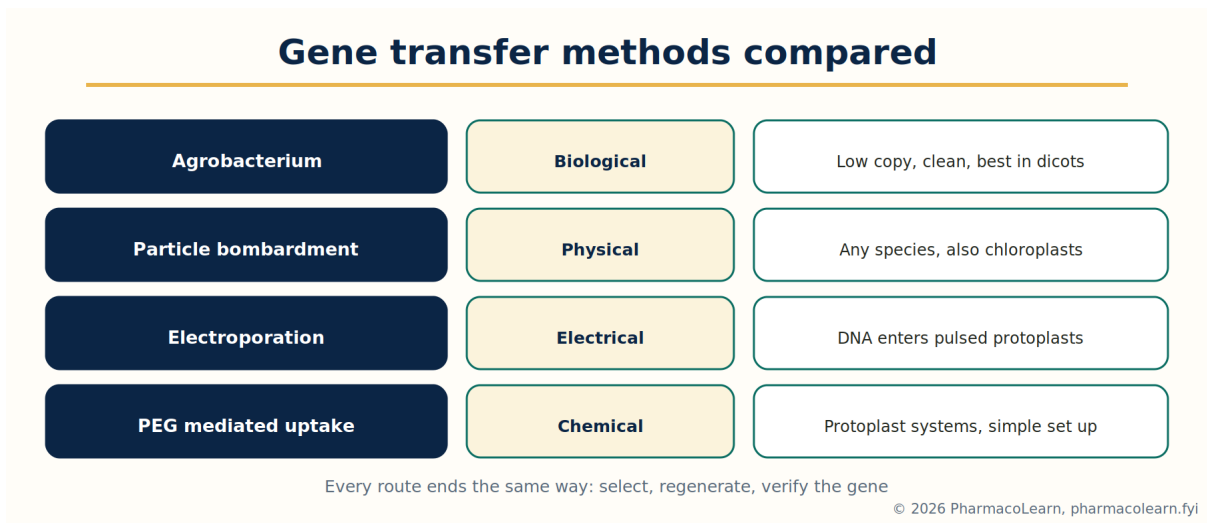


Figure: Transformation methods compared. Every route ends at the same obligations: select the transformed cells, regenerate the plant, and verify the gene and its expression.

4.5 Methods used in gene identification, localization and sequencing of genes

Before a gene can be transferred, improved or even discussed precisely, it must be found, placed and read, and this section teaches the three verbs of the syllabus in order. Identification asks which DNA is the gene for a trait or enzyme. The classical routes complement each other: screen a DNA library with a probe, a labelled piece of related DNA that hybridises to its match; use the protein, purifying the enzyme and working back from its amino acid sequence to the gene; use a mutant or a differing line and find the DNA that differs; or amplify the gene directly by PCR from a known related sequence, Section 4.6. Hybridisation, the binding of complementary single strands, is the working principle under most of these, in colony and plaque screening and in the blot techniques that detect a specific DNA or RNA in a mixture. Localization asks where the gene sits: on which chromosome and at what position, established by genetic mapping, the measurement of recombination distances between the gene and known markers, and by physical methods, hybridisation of a labelled probe directly to chromosome preparations, which shows the site by sight. Sequencing asks for the exact base order, read by the standard chain termination method and its automated descendants, in which synthesis is stopped base by base and the fragment sizes reveal the sequence. Once read, a sequence is compared against databases of known genes: resemblance to a characterised enzyme gene from another species is often the first functional identification of a newly read medicinal plant gene. The three verbs chain together in practice: identify a candidate, localise and clone it in full, sequence it, and only then does the gene become a tool the rest of the subject can use.

4.6 Application of PCR in plant genome analysis

The polymerase chain reaction, PCR, is the in vitro amplification of a chosen DNA segment: a cycle of heating to separate the strands, cooling to let two short primer sequences bind at the two ends of the target, and extension by a heat stable DNA polymerase, repeated many times so the segment between the primers doubles each cycle and becomes abundant enough to detect, clone or sequence. Its applications in plant genome analysis, the syllabus phrase, should be given as a working list tied to this subject. Detection and verification: confirming that a transferred gene is present in a regenerated plant, the routine verification

of Sections 2.11 and 4.4. Identification work: amplifying a gene or a diagnostic region from a medicinal plant for cloning and sequencing, Section 4.5. Identity and relationship studies: PCR with primers for variable regions, and PCR based marker methods that reveal differences between plants without prior sequence knowledge, are used to distinguish species, varieties and chemotypes, to check the authenticity of drug material and the fidelity of micropropagated lines, and to build the marker data that genetic mapping and localization consume. Expression study: PCR performed on DNA copied from RNA reports whether a pathway gene is switched on in a tissue or a culture, connecting genome analysis back to the regulation questions of Units 1 and 3. Genome survey at scale: amplification across many loci supports diversity assessment of medicinal plant collections and germplasm, guiding conservation and parent selection. The common thread to state in closing is economy: PCR turns an invisible trace of DNA into a workable quantity, which is why it appears at every stage of genome analysis, from the first screen of a transformant to the authentication of the drug plant itself.

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