

Lecture 4. Plant Genome Transformation Technologies. Methods for Introducing New Genes into Plants, Vector Systems, and Selection Markers

Introduction

Plant genome transformation is one of the key technologies of modern plant biotechnology and genetic engineering. It involves the introduction of new genetic material into plant cells in order to modify gene expression, study gene function, or produce plants with new and useful characteristics. Genetic transformation has become an important tool for both fundamental research and practical agriculture because it allows researchers to work directly with specific genes rather than relying only on naturally occurring genetic variation and conventional breeding. Through plant transformation, scientists can investigate the molecular mechanisms controlling plant growth, development, metabolism, reproduction, and responses to environmental factors. The technology can also be used to develop plants with resistance to pathogens and pests, improved tolerance to environmental stresses, modified nutritional properties, or altered production of biologically active compounds. Plant transformation is closely connected with tissue culture because the introduction of genetic material is usually followed by the selection of transformed cells and regeneration of complete plants. A typical transformation process includes identification of a target gene, construction of a suitable genetic vector, delivery of genetic material into plant cells, selection of transformed cells, regeneration of plants, and molecular confirmation of the genetic modification.

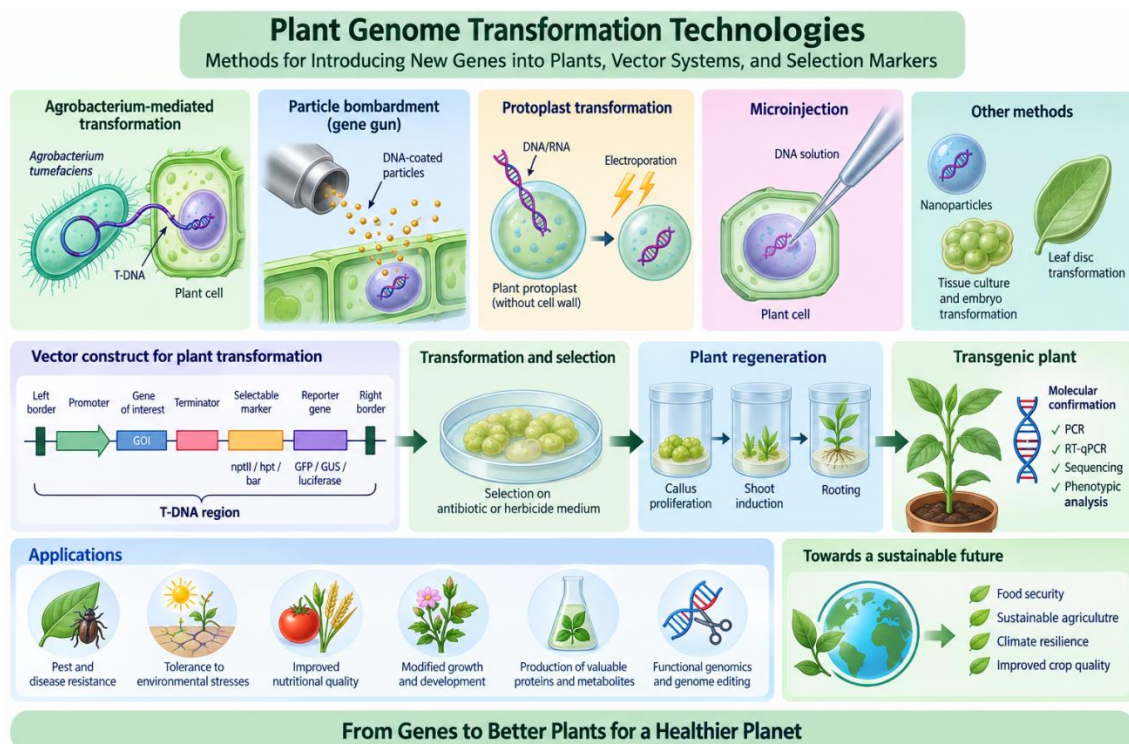


Figure 1. Graphical Abstract of Plant Genome Transformation Technologies: Methods for Introducing New Genes, Vector Systems, Selection Markers, and Plant Regeneration

Principles of Plant Genetic Transformation

Plant genetic transformation can be either stable or transient. In stable transformation, introduced DNA becomes integrated into the plant genome and is maintained during cell division. If transformed cells are successfully regenerated into fertile plants, the introduced genetic information may be transmitted to subsequent generations. Stable transformation is therefore particularly important for producing genetically modified plant lines and studying heritable characteristics. In transient transformation, genetic material enters plant cells and is expressed for a relatively short period without necessarily becoming permanently integrated into the genome. This approach is useful for rapid investigation of promoter activity, protein localization, gene regulation, and interactions between proteins. Regardless of the type of transformation, an effective genetic construct normally contains several functional elements. The gene of interest determines the biological characteristic that researchers want to introduce or investigate. A promoter controls transcription of the gene, while a terminator provides signals necessary for appropriate termination of transcription and RNA processing. The construct frequently contains a selectable marker that helps distinguish transformed cells from non-transformed cells and may also include a reporter gene that provides a detectable signal. Thus, successful plant transformation depends not only on introducing DNA into a cell but also on ensuring appropriate regulation and expression of the introduced genetic information.

Methods for Introducing New Genes into Plants

Methods of plant transformation can generally be divided into biological and direct methods. One of the most widely used biological approaches is *Agrobacterium*-mediated transformation. *Agrobacterium tumefaciens* is a soil bacterium that naturally possesses the ability to transfer a specific DNA region into plant cells. This natural mechanism has been adapted for genetic engineering. The bacterium contains a large Ti, or tumor-inducing, plasmid that includes a region known as T-DNA. During natural infection, T-DNA can be transferred from the bacterial cell into a plant cell and integrated into plant nuclear DNA. In biotechnology, genes responsible for the natural disease-causing effects are removed, while the DNA-transfer system is retained. Researchers can then place a desired gene within the T-DNA region so that the bacterial transfer mechanism delivers this engineered genetic material into plant cells. Virulence, or *vir*, genes provide proteins involved in processing and transferring T-DNA, but these genes themselves are generally not incorporated into the plant genome. *Agrobacterium*-mediated transformation has become especially important because it can provide efficient transformation and relatively stable integration in many plant species. Although it was historically used mainly for dicotyledonous plants, improvements in transformation and tissue-culture methods have expanded its use to many monocotyledonous crops.

Another important approach is particle bombardment, also called biolistic transformation or the gene gun method. In this method, microscopic particles carrying genetic material are physically introduced into plant cells and tissues. Some of the delivered DNA reaches cells capable of expressing the introduced genes and, under appropriate conditions, may become stably integrated into the plant genome. Particle bombardment can be useful for plant species and tissues that are difficult to transform using biological systems. It has also been applied to transformation of plant organelles such as chloroplasts. However, physical delivery may damage some cells, and DNA integration can sometimes involve multiple copies or complex

arrangements. Plant protoplasts provide another important experimental system. A protoplast is a plant cell whose cell wall has been removed, making the plasma membrane more accessible to introduced molecules. Genetic material can therefore be delivered into protoplasts for transient expression and genome-editing studies. Electroporation can also be used, particularly with protoplasts. Short electrical pulses temporarily increase membrane permeability and allow genetic material to enter cells. Other approaches include microinjection and emerging delivery technologies based on nanoscale carriers. The choice of method depends on the plant species, genotype, tissue, purpose of the experiment, and ability of transformed cells to regenerate into complete plants.

Vector Systems and Gene Expression

Vectors are essential components of plant genetic engineering because they carry the genetic material that researchers want to introduce into plant cells. Modern *Agrobacterium*-mediated transformation frequently uses binary vector systems. In these systems, the T-DNA containing the gene of interest is physically separated from many of the bacterial genes responsible for DNA transfer. One component carries the engineered T-DNA, while another provides the necessary virulence functions. This arrangement makes genetic constructs easier to manipulate and allows researchers to introduce different genes into plants using the same basic transformation system. A typical plant transformation vector contains T-DNA border sequences, a promoter, the gene of interest, a terminator, and one or more genes used for selection or detection. Additional sequences allow the vector to be maintained and manipulated in bacterial cells during molecular cloning. The promoter is particularly important because it determines where, when, and how strongly a gene is expressed. Constitutive promoters can support expression in many tissues, whereas tissue-specific promoters restrict expression mainly to particular organs or developmental stages. Inducible promoters respond to specific environmental, chemical, or biological signals. Appropriate promoter selection is therefore an important part of genetic construct design because strong expression throughout the entire plant is not always desirable.

Selection Markers, Reporter Genes, and Plant Regeneration

Only a proportion of cells exposed to a transformation procedure successfully acquire the desired genetic material. Selectable marker genes are therefore used to distinguish transformed cells from non-transformed cells. A selectable marker provides a characteristic that allows transformed cells to survive or grow under defined selection conditions. Marker systems historically used in plant biotechnology include genes such as *nptII*, *hpt*, and *bar/pat*. Selection makes it possible to identify populations of cells that are more likely to contain the genetic construct. Reporter genes have a different purpose: instead of primarily providing a survival advantage, they generate an observable or measurable signal. Common reporter systems include GUS, GFP, and luciferase. GFP produces fluorescence, GUS provides a detectable enzymatic signal, and luciferase generates bioluminescence. These systems are useful for studying promoter activity, transformation efficiency, gene-expression patterns, and protein localization. Modern biotechnology is also interested in marker-free transformation strategies because selectable marker genes may not be required after transformed plants have been obtained and characterized.

After transformation and selection, transformed cells must often be regenerated into complete plants. This process depends on plant tissue culture and the principle of cellular totipotency, which refers to the ability of certain plant cells to generate a complete organism under appropriate conditions. Regeneration may involve cell proliferation followed by the formation of shoots and roots, although the exact process varies considerably among species. This stage represents one of the major challenges in plant biotechnology because some species and cultivars regenerate readily whereas others are highly resistant to tissue-culture procedures. Once plants have been regenerated, molecular analyses are required to confirm transformation. PCR can be used to detect the presence of target DNA sequences, while sequencing can confirm their identity. Analysis of RNA can determine whether the introduced gene is transcribed, and protein-based methods can determine whether the corresponding protein is produced. Phenotypic analysis is then used to establish whether the genetic modification produces the expected biological characteristic. Reliable evaluation therefore combines molecular, biochemical, genetic, and phenotypic evidence.

Applications and Future Perspectives

Plant genome transformation has numerous applications in agriculture and biological research. It can be used to investigate genes associated with resistance to insects and plant pathogens, tolerance to drought, salinity and other environmental stresses, nutritional composition, plant architecture, flowering, fruit development, and post-harvest characteristics. Genetic transformation can also modify metabolic pathways so that plants or plant cell cultures produce valuable proteins, enzymes, metabolites, and other biological compounds. An especially important modern application is genome editing. Technologies such as CRISPR/Cas allow targeted modification of specific genomic sequences, but efficient genome editing still depends on the successful delivery of editing components into plant cells. Consequently, *Agrobacterium*-mediated transformation, particle-based delivery, and protoplast systems continue to play important roles even as genome-editing technologies develop. Plant transformation and genome editing are therefore complementary technologies rather than completely separate approaches.

The development and application of genetically modified and genome-edited plants also require consideration of biosafety, environmental effects, and ethical issues. Important questions include the stability of genetic modifications, possible gene flow to related plants, effects on non-target organisms and biodiversity, and the consequences of particular traits for agricultural management. Food and feed applications may additionally require assessment of composition, nutritional characteristics, toxicity, and allergenic potential according to relevant regulatory requirements. Future plant transformation technologies are expected to become more precise, efficient, and applicable to a wider range of plant species. Advances in genomics, bioinformatics, synthetic biology, genome editing, tissue culture, and molecular delivery systems are helping researchers develop approaches that reduce unnecessary genetic changes and improve control over gene expression. New strategies are also being investigated for delivering RNA, proteins, and genome-editing components without permanent incorporation of foreign DNA. Together, these developments will strengthen the role of plant biotechnology in crop improvement, sustainable agriculture, food security, and adaptation to changing environmental conditions.

Conclusion

Plant genome transformation is a fundamental technology of modern plant bioengineering that enables genetic material to be introduced into plant cells for research and crop improvement. The major approaches include *Agrobacterium*-mediated transformation, particle bombardment, protoplast-based methods, electroporation, and other physical or biological delivery systems. Successful transformation requires an appropriate vector containing regulatory elements, a gene of interest, and suitable selection or reporter systems, followed by selection of transformed cells, plant regeneration, and molecular confirmation. The development of binary vectors and efficient tissue-culture methods has significantly expanded the possibilities of plant genetic engineering, while modern genome-editing systems such as CRISPR/Cas are making genetic modification increasingly targeted and precise. Together, transformation and genome-editing technologies provide powerful tools for understanding plant biology and developing crops with useful agricultural, nutritional, and environmental characteristics.