Chapter 1
Plant Nuclear Transformation

John J. Finer

1.1 Introduction to Plant Transformation

"Transformation" is most simply defined as a "change". In the plant biotechnology community, transformation can be a little more precisely defined as the process of DNA introduction into a plant cell, leading to a permanent change in the genetic makeup of the target cell and its derivatives.

The ability to produce whole plants from transformed plant cells, first reported by Horsch et al. (1985), has revolutionized the plant sciences and changed the face of the planet, through the success and rapid adoption of genetically modified crops. Although the transformation process itself was initially limiting, all crops of major interest have been successfully transformed and many if not most transformation technologies are considered routine. Some crops do remain a little recalcitrant to transformation and improvements in the methods for production of stably-transformed plants are still needed. The current limitations in the production of transgenic plants for both basic research and commercial application include more efficient production of transformed plants and obtaining more predictable insertion and expression of the introduced DNA.

1.1.1 DNA Introduction Basics

DNA introduction can impact and modify any of the organelles within the plant cell that also contain DNA. Suitable targets include the nucleus, plastid and mitochondrion. Plastid transformation is presented in the next part of this chapter while this

J.J. Finer
Department of Horticulture and Crop Science, Plant Molecular Biology and Biotechnology Program, Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, USA
e-mail: finer.1@osu.edu

F. Kempken and C. Jung (eds.), *Genetic Modification of Plants*,
Biotechnology in Agriculture and Forestry 64,
DOI 10.1007/978-3-642-02391-0_1,

portion of the chapter focuses exclusively on nuclear transformation. Transformation of the mitochondrion has been reported for some organisms (Johnston et al. 1998) but has not yet been reported for higher plants.

For (nuclear) transformation to be successful, DNA must first be introduced into the target cell. The DNA molecule is sufficiently large so that a physical entry point through the cell wall and cell membrane must be established and this can compromise the health of the targeted cell. After passage through the plant cell wall and membrane, the introduced DNA must then proceed to the nucleus, pass through the nuclear membrane and become integrated into the genome. It is believed that the introduced DNA can function for a short time in the nucleus as an extrachromosomal entity, but integration into the genetic material of the target cell is necessary for long-term functionality and expression.

To recover a transgenic plant, the single cell that is the recipient for DNA introduction must be capable of either forming a whole plant or contributing to the zygote, through either the pollen or the egg. Therefore, successful recovery of transgenic plants largely relies on the ability to either transform the pollen/egg directly (Ye et al. 1999) or the non-gametic cells (somatic cells; Horsch et al. 1985), which must be subsequently manipulated to form whole plants. In most cases, plant transformation relies heavily on the ability of the plant cells and tissues to form whole plants through the tissue culture process. Efficiencies, or at least the ease of transformation, would be tremendously increased if regeneration processes were improved. As things now stand, the methodologies for transformation that are described in this book are consistent and workable but improvements are always desirable.

1.2 Transient Expression

Transient expression is exactly what the phrase suggests: a short-term expression of the introduced DNA(s). Directly following the introduction of DNA into the nucleus, that DNA starts to function. Transient expression is usually studied using scorable marker genes, which report their expression via direct production of a detectable colored/fluorescent compound or an enzyme that can convert a non-pigmented substrate into a pigmented form. The most commonly used scorable markers are β-glucuronidase (GUS) from *Escherichia coli* which converts a colorless substrate into a blue form (Jefferson 1987) and the green fluorescent protein (GFP) (Chalfie et al. 1994) which fluoresces green upon excitation by high-intensity UV or blue light. Although expression from the *gus* gene is relatively simple and inexpensive to detect, the GUS assay itself is toxic and can therefore only be used for single time-point expression assays. In contrast, proper analysis of expression of the *gfp* gene requires costly instrumentation but gene expression can be continually observed in the target tissues over time.

Transient expression represents the first indication of successful gene introduction and function. In the development of new DNA introduction methodologies,

observation of a single blue GUS-expressing cell or a few GFP spots is usually all that is needed to suggest further investigations of an approach. Transient expression in a cell should be very clear following visualization of GUS or GFP. Expression is most often limited to the targeted cells and a demarcation of expressing and non-expressing areas should be apparent. Faint or diffuse expression of the marker genes (if regulated by the appropriate promoter) is usually an indication of improper assay conditions.

Most transformation procedures were developed based on optimization of DNA delivery using transient expression analyses. Transient expression for GUS is typically observed 24 h post-introduction (Klein et al. 1988) while GFP expression can be observed as early as 1.5 h after delivery (but peak expression usually occurs at 8–24 h; Ponappa et al. 1999). Although studies of transient expression itself are not common, these studies do provide information on the early fate of the introduced DNA.

1.2.1 Optimization of Transient Expression

Since transient expression is a direct measure of successful DNA introduction and function, development of methods to improve transient expression has often been used as a means of optimizing the transformation process itself (Klein et al. 1988). This approach has been quite useful and successful over the years. However, transient expression is only a measure of successful short-term transgene expression and it may not always perfectly reflect the ability of the cells to integrate the introduced DNA to generate stable events. As stated earlier, as the DNA molecule is so large, the process of DNA introduction itself requires that the integrity of the cell be compromised in some way. Target tissues and cells can therefore be sufficiently damaged by the DNA delivery process so that they express the transgene at high levels but not survive over the long term. This point of diminishing returns cannot be precisely defined for the different systems but it does exist. Optimization of transient expression is quite useful for the initial development of transformation methods but the efficiency of stable transformation and stability of transgene expression should be the ultimate goals of most transformation efforts.

1.2.2 Transient Expression to Study Gene Expression and Stability

In addition to using transient expression to optimize transformation and DNA introduction methods, this type of rapid transgene expression can also be used to facilitate speedy analysis of factors that influence the strength and stability of transgene expression (Sheen 2001; Dhillon et al. 2009). Once transient expression is optimized and standardized for a specific target tissue, the effects of factors that influence the level and profile of transgene expression can be reliably determined.

Quantification of transient expression, required for this type of analysis, involves either the extraction of the gene product from the targeted tissues (Klein et al. 1987) or the use of image analysis for continual monitoring of *gfp* gene expression over time (Finer et al. 2006). Tracking of GFP expression coupled with image analysis has tremendous advantages over tissue extraction as gene expression in the same piece of tissue can be followed over time.

Transient expression analysis has been utilized to study the relative strengths of different promoters and promoter fragments (Chiera et al. 2007) and to evaluate genes that modulate the introduced transgene via gene silencing (Chiera et al. 2008). Surprisingly, promoter analysis using transient expression does not appear to reflect promoter tissue-specificity (Finer, unpublished data), which suggests that large amounts of pre-integrative DNAs do not behave exactly like single- or low-copy integrated genes. However direct promoter strength comparisons do appear to be transferable from transient expression studies to expression in stably transformed tissues (Hernandez-Garcia et al. 2009). Promoter isolation and evaluation could increase tremendously with the increased availability of genome sequences from a number of different plants. Since the production of stably transformed plants can take from weeks to months, the use of transient expression may be desirable when rapid promoter analysis is needed.

Transient expression has been used to evaluate factors that influence the stability or consistency of gene expression (Dhillon et al. 2009). As gene expression variability among different events is a significant limitation in the production of transgenics, this approach may be quite useful as a preliminary evaluation tool for transgene stabilization work. The final determination of factors that modulate transgene expression must ultimately be made only following introduction to plant cells for stable transformation.

1.3 Agrobacterium Background

Agrobacterium tumefaciens is a soil-borne bacterium that causes crown gall disease in plants. Infected plants display a gall on the stem which is composed of proliferating plant cells that were transformed with bacterial DNA. The wild-type bacterial pathogen has the special ability to invade accessible areas of the target plant, adhere to certain types of plant cells and insert some of its own DNA (Bevan and Chilton 1982). This DNA is coated with different bacterial encoded proteins, which protect the DNA from degradation, direct transport to the nucleus and assist with the integration of bacterial DNA into the plant genomic DNA. The bacterial DNA that is transferred (T-DNA) is located in the bacterial cell on a native plasmid, called the tumor-inducing plasmid (Ti plasmid). In the wild-type bacterium, the T-DNA contains genes for synthesis of nitrogen-rich opines (which are metabolized by associated bacteria) and plant hormones, which cause rapid cell proliferation leading to the formation of galls.

This brief background on *Agrobacterium* is significant as the current era of plant biotechnology was born after Mary-Dell Chilton (Bevan and Chilton 1982) and Jeff Schell (Zambryski et al. 1983), along with scientists at Monsanto (Horsch et al. 1985), found that they could replace the native opine- and hormone-producing genes in the T-DNA with any gene(s) of interest and introduce those genes into plant cells. With the opine- and hormone-producing genes removed, the T-DNA becomes "disarmed". A large number of additional discoveries enabled *Agrobacterium* to become the transformation vehicle of choice for many if not most plant transformation systems.

1.3.1 A String of Improvements for Agrobacterium

The use of *Agrobacterium*, in its original form, for the transformation of plant cells was both inefficient and unwieldy. First, the Ti plasmid was difficult to manipulate for introduction of genes of interest as it was so large. In addition, the bacterium was originally only able to infect and transform a limited number of plants and even specific cells within those plants. Due to perceived host-range limitations, grasses and monocots in general were thought to be unresponsive to *Agrobacterium*-mediated transformation. Last, wounding of the target tissue was deemed absolutely necessary as an entry point for the bacteria.

To make DNA introductions and manipulations simpler, binary vector systems were developed for use with *Agrobacterium* (DeFramond et al. 1983; Bevan 1984). The wild-type Ti plasmid contains both the T-DNA and a virulence (vir) region that encodes for genes involved in the T-DNA transfer machinery. Binary vectors allow for the separation of function on different plasmids; the Ti plasmid retains the vir region (T-DNA is removed) and the modified T-DNA is placed on the smaller binary vector, which can be more easily manipulated in the laboratory. The *vir* genes act *in trans*, leading to the processing of the T-DNA from the binary vector, for delivery to the targeted plant cells.

The host range limitations, originally associated with this biological pathogen and vector, have been largely overcome. As with most pathogens, different pathovars exist, which show different infectivity on different plants and cultivars of plants. Various *Agrobacterium* strains, which were selected for their high virulence, are now routinely used for plant transformation. The single advance, which had the greatest impact on increasing the host range for *Agrobacterium*, was the discovery that wounded plant tissues produced acetosyringone (Stachel et al. 1985), which subsequently induced some of the *vir* genes to initiate the T-DNA transfer process. Acetosyringone is now routinely included in the plant/bacterial co-culture medium at 100–200 μM. This chemical inducer of T-DNA transfer shows no deleterious effects on plant growth and development and it is always best to include this compound during co-culture, rather than risk the chance of obtaining inefficient transformation. As an alternative to including acetosyringone, *Agrobacterium* has been generated which constitutively expresses the *vir* genes (Hansen et al. 1994), which can give similar results.

Springer

http://www.springer.com/978-3-642-02390-3

Genetic Modification of Plants
Agriculture, Horticulture and Forestry
Kempken, F.; Jung, C. (Eds.)
2010, XXVII, 675 p., Hardcover
ISBN: 978-3-642-02390-3