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Risk Assessment Reference: Methods of Plant Genetic Modification

Introduction

Plant genetic modification, also called plant transformation, is the process of introducing a new DNA fragment into the genome of a plant. Several methods are available to generate genetically modified (GM) plants. These have been described in a number of articles, e.g. Hooykaas, 2010; Barampuram and Zhang, 2011; Rivera et al., 2012; Peyret and Lomonossoff, 2015; Hwang et al., 2017; Zhao et al., 2017; Cunningham et al., 2018.

Transformation methods can be divided into two major categories: indirect and direct DNA delivery. In the indirect method, the new DNA is introduced into the plant cell via bacteria, usually *Agrobacterium tumefaciens* or less commonly *Agrobacterium rhizogenes*. In the direct transformation methods, the new DNA is introduced without an intermediate host. The most commonly used direct transformation method is biolistic transformation (reviewed by Rivera et al. (2012)). These two transformation methods are outlined below.

Agrobacterium-mediated transformation

Agrobacterium tumefaciens is a common soil bacterium that naturally causes gall formation on a wide range of plant species, including most dicotyledonous and some monocotyledonous species (Van Larebeke et al., 1974). The gall is induced by transfer of hormone-producing genes from the bacterial cell into the plant genome. The genes are carried on a circular DNA molecule found within the bacterial cell called a Tumour-inducing (Ti) plasmid. During the infection process, only a section of the Ti plasmid known as the Transfer DNA (T-DNA) is transferred to the plant. The infection and T-DNA transfer process of *A. tumefaciens* has been extensively studied. This natural process has been used to facilitate genetic modification of plants.

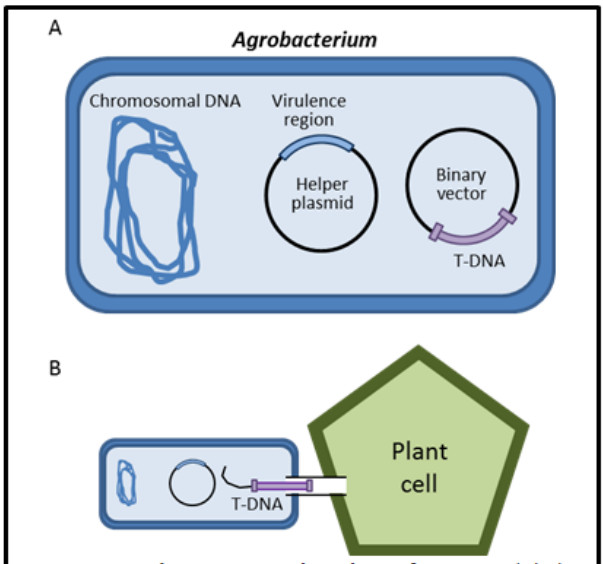

Figure 1. *Agrobacterium*-mediated transformation. (A) The *Agrobacterium* cell contains two plasmids (circular DNA). The disarmed binary vector carries the T-DNA that will be transferred to the plant cell. The helper plasmid contains the virulence genes that allow the infection of the plant cell and the delivery of the T-DNA. (B) Cartoon depicting the transfer of the T-DNA to the plant cell.

A. tumefaciens Ti plasmids have been produced that lack the genes responsible for gall formation (disarmed plasmids; Figure 1). Genes to be inserted into the plant are put into the T-DNA section of these disarmed plasmids.

A. tumefaciens cells carrying such plasmids cannot produce a gall in an infected plant but will transfer the T-DNA sequence carrying the genes of interest into the plant cell where they stably integrate into the plant genome (Bevan, 1984; Klee and Rogers, 1989). Although most monocotyledoneous plants are not natural hosts of *A. tumefaciens* (De Cleene and De Ley, 1976), recent technical developments

have enlarged the range of *Agrobacterium*-susceptible hosts, so that they now include dicotyledonous and monocotyledonous plants (Sood et al., 2011; Koh et al., 2015).

Agrobacterium-mediated transformation usually results in one or a few T-DNA insertions into the plant genome. Small segments of the T-DNA flanking sequence or *A. tumefaciens* chromosomal sequence may also be transferred into the plant genome at a low frequency (Smith, 1998; Ülker et al., 2008). The likelihood of *A. tumefaciens* DNA having an impact on GM plants is small because *Agrobacterium* chromosomal genes do not contain regulatory elements required for expression in plants, and are therefore unlikely to be expressed.

Biolistic transformation (particle bombardment)

In this technique, DNA is delivered into plant cells on small tungsten or gold carrier particles, approximately 2 microns in diameter. The particles are coated with the gene(s) of interest and fired into plant cells or tissues, usually using pressurised helium (Figure 2). Some of the particles penetrate the cell nucleus, where the introduced genetic material is incorporated into nuclear DNA (Sanford, 1990). Biolistic transformation often results in multiple DNA insertions at different sites within the genome (Rivera et al., 2012).

In recent years, biolistic transformation has become a very common method to genetically modify plants, and has been shown to be applicable to virtually all species investigated. It can also be used to deliver DNA to specific parts of plant cells, e.g. chloroplasts (Barampuram and Zhang, 2011).

The process of plant transformation

Transformation can be achieved using a variety of plant tissues, e.g. leaf discs, embryos and protoplasts. After the DNA delivery step, a whole plant must be regenerated from the transformed plant tissues. In most cases, plant regeneration involves a tissue culture process (Figure 2). In this process, plant tissues are transferred to a synthetic medium containing a selective agent such as an antibiotic to eliminate untransformed cells [1] as well as nutrients and hormones to promote the growth of plants from single transformed cells. Each of the regenerated plantlets represents a GM line or event. In each GM line the new DNA fragment would have integrated randomly at a different position in the plant genome.

All regenerated plants undergo a selection process that involves a phenotypic and molecular evaluation (Koh et al., 2015). At a phenotypic level, GM lines showing the trait of interest and without any undesired effects are selected. These selected GM lines are characterised at a molecular level, which includes determining the number of inserts they carry and their position within the genome. Selected GM lines are also usually backcrossed to their non-GM parent to eliminate any unintended effects of the transformation process (see below).

Unintended effects of plant transformation

The process of plant transformation may induce other changes to the plant DNA. Unintended effects of the process of plant transformation are insertional effects and somaclonal variation.

Insertional effects. The insertion of the new DNA into the genome can lead to insertions, deletions and rearrangements (Latham et al., 2006; Wilson et al., 2006; Schnell et al., 2015). Additional DNA can be inserted in the plant genome. This DNA may come from the plant genome, the inserted DNA or it can simply be random filler DNA. Small deletions in the area flanking the site of insertion are also common. These deletions are typically less than 100 bp, although larger deletions have been occasionally observed. Transformation can also result in rearrangements of both the introduced DNA

[1] Information on use of antibiotics and antibiotic selection markers in GM plants can be found in the risk assessment reference document *Marker Genes in GM Plants* available on the OGTR website.

and the host plant DNA. For instance, chromosomal translocations have been documented, where the flanking genomic DNA on either side of the introduced DNA mapped to two different chromosomes. The new DNA may also knock out genes when integrating into the genome or affect the expression of neighbouring genes (Latham et al., 2006; Schnell et al., 2015). These alterations to the plant DNA are collectively known as insertional effects.

Somaclonal variation. The tissue culture stage can also cause DNA substitutions, insertions, deletions, rearrangements and changes in chromosome number (Latham et al., 2006; Schnell et al., 2015). This is known as somaclonal variation. These newly generated mutations arise as a result of the tissue culture process and are not related to the genetic transformation. Horticultural species that are propagated by tissue culture can also display somaclonal variation. The mutagenicity of tissue culture has been attributed to the stress the plant cells suffer during this process (Krishna et al., 2016). Stressing conditions include wounding, exposure to chemicals that prevent growth of bacteria, and high concentration of plant growth regulators in the media. Different factors affect the frequency of somaclonal variation (Krishna et al., 2016), including the plant species, the mode of regeneration, the length of culture period, and the culture environment. In general, the longer the period of tissue culture and the more disruptive the process, the more mutations are induced. For instance, the production of plants via axillary branching does not normally result in the production of somaclonal variants, while cultures that go through a callus phase promote a higher mutation rate (Leva et al., 2012; Krishna et al., 2016). Somaclonal variation results in a similar spectrum of genetic variation as mutagenesis by chemical or physical agents (Krishna et al., 2016). In fact, it is employed to develop novel cultivars in breeding programs. The extent of somaclonal variation showed by the GM plants will depend on the type of tissue culture technique used to regenerate them.

Insertional effects and somaclonal variation may lead to unintended traits in the plant if they affect the expression of endogenous genes or create novel proteins (Schnell et al., 2015). If unintended traits arise from changes in gene expression, these may or may not be harmful.

The range of DNA changes observed in GM plants is similar to other genetic changes that occur spontaneously in plants and during conventional breeding (Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health, 2004; Bradford et al., 2005; Weber et al., 2012; Steiner et al., 2013; Ladics et al., 2015; Schnell et al., 2015). Differences in gene expression and metabolic composition are typically greater between conventionally bred cultivars than they are between a GM plant and its non-GM parent (Baudo et al., 2006; Batista et al., 2008; Clarke et al., 2013; Schnell et al., 2015).

It is important to bear in mind that new plant varieties developed by both conventional breeding and genetic modification undergo a selection process in which any undesired effects are eliminated before reaching a commercial product (Ladics et al., 2015; Schnell et al., 2015).

Conclusion

Despite the widespread employment of these methods of plant transformation, there have been no reports of adverse effects on human health and safety or the environment as a result of their use. Nevertheless, irrespective of which method of transformation was used, the potential for adverse unintended effects as a result of gene technology is assessed on a case-by-case basis, in the context of the proposed dealings, in each risk assessment for release of a GM plant

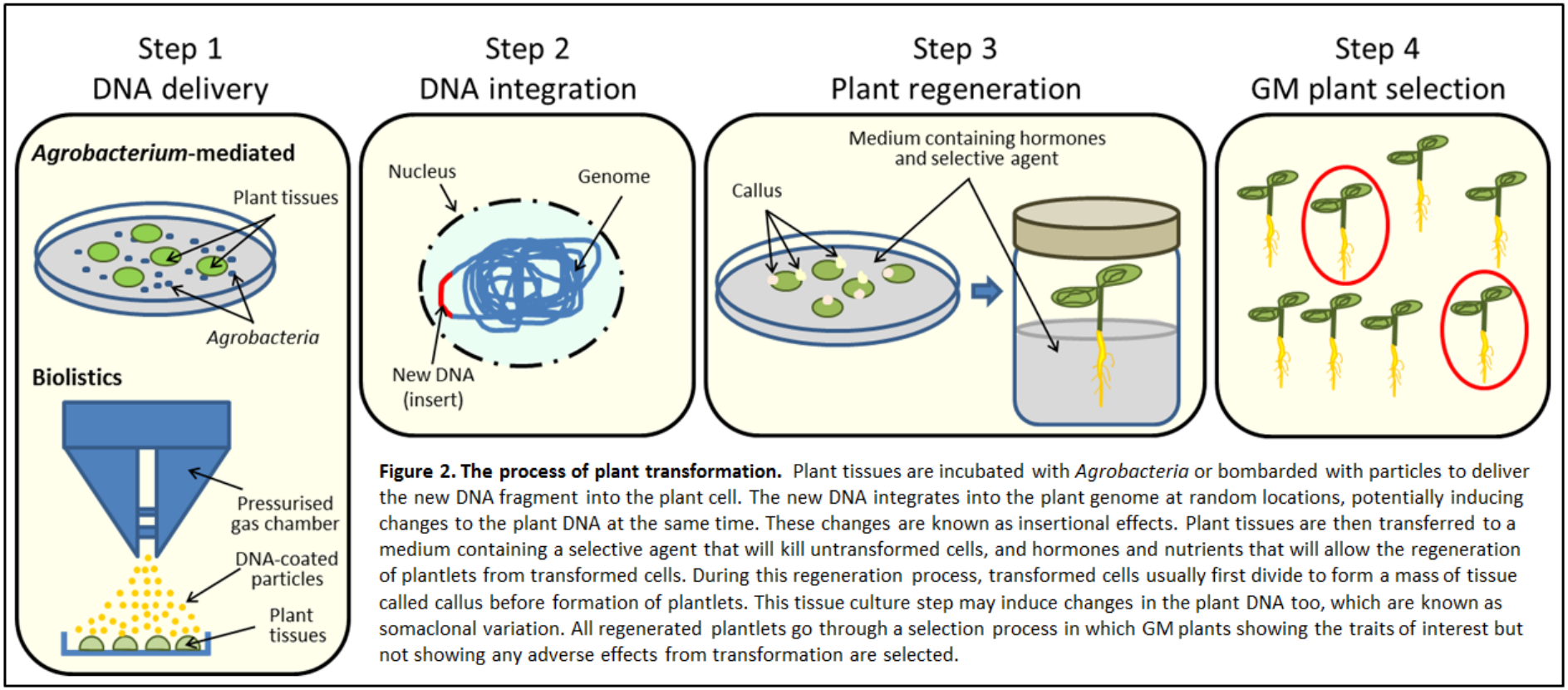

Figure 2. The process of plant transformation. Plant tissues are incubated with *Agrobacteria* or bombarded with particles to deliver the new DNA fragment into the plant cell. The new DNA integrates into the plant genome at random locations, potentially inducing changes to the plant DNA at the same time. These changes are known as insertional effects. Plant tissues are then transferred to a medium containing a selective agent that will kill untransformed cells, and hormones and nutrients that will allow the regeneration of plantlets from transformed cells. During this regeneration process, transformed cells usually first divide to form a mass of tissue called callus before formation of plantlets. This tissue culture step may induce changes in the plant DNA too, which are known as somaclonal variation. All regenerated plantlets go through a selection process in which GM plants showing the traits of interest but not showing any adverse effects from transformation are selected.

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