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## Review

# Manipulation of epigenetic factors and the DNA repair machinery for improving the frequency of plant transformation

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## ABSTRACT

Plant genetic engineering involves the introduction of foreign DNA into the plant genome in order to enhance/modify plant traits. In transgenic plants, it is difficult to achieve stable and predictable transgene expression over subsequent generations. Largely, this is due to the lack of critical understanding of plant perception and response to the artificially introduced foreign DNA. Recent reports have revealed components of the epigenetic module that may affect transgene stability at both pre- and post-integration steps. Furthermore, the integration of the transgene has been shown to be strictly dependent on the DNA repair machinery. In this review, we briefly summarize genetic and epigenetic factors whose manipulation can enhance the efficiency of plant transformation and the quality of genetically engineered transgenic plants.

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## 1. Introduction

Plant genetic engineering has emerged as a vital tool of contemporary biotechnology. The ability to introduce a foreign

gene into the plant genome has resulted in the development of a number of transgenic crops with beneficial traits (Collinge et al., 2010; Ahmad et al., 2012). Nevertheless, the improvement of economically important crops necessitates a stable and predictable transgene expression that is usually hard to achieve in the field conditions because it requires a tremendous amount of labor and time to select for a desired transgenic line (Curtin et al., 2012). Mainly, this is due to three major challenges that are still to be addressed in plant biotechnology: (i) many important crop species remain recalcitrant to tissue culture regeneration; (ii) the transformation frequency – the number of transgenic plants (transgenics) generated in a single transformation round – is low; (iii) a low frequency of integration of the transgene into a

*Abbreviations:* C-NHEJ, canonical NHEJ; DSBs, DNA double-strand breaks; gDNA, genomic DNA; GT, gene targeting; HR, homologous recombination; NHEJ, non-homologous end joining; PTGS, posttranscriptional gene silencing; RNAi, RNA interference; *rat*, resistant to *Agrobacterium*-mediated transformation; siRNA, small interfering RNA; T-DNA, transferred DNA; *vir*, virulence

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desired position of the host genome to obtain plants with a predictable transgene expression pattern (Barampuram and Zhang, 2011; Husaini et al., 2011). Although a tissue culture step is of great importance for plant transgenesis, in this review, we will focus on the latter two challenges.

Although a great number of DNA delivery methods have been developed, the *Agrobacterium*-mediated plant transformation method still remains to be the primary tool for stable transformation of many dicotyledonous (dicot) and some monocotyledonous (monocot) crops (Hiei et al., 1994; Leelavathi et al., 2004; Hu et al., 2005). In fact, the inability to achieve high frequencies of transformation events mediated by *Agrobacterium* in monocots and recalcitrant plant species has prompted the development of specific direct DNA transfer methods (Barampuram and Zhang, 2011; Rivera et al., 2012). *Agrobacterium tumefaciens* belongs to the genus *Agrobacterium* that includes mostly saprophytic soil borne bacterial species which inhabit the rhizosphere (Escobar and Dandekar, 2003; Păcurar et al., 2011). *Agrobacterium* has a natural ability to transfer a portion of a tumor-inducing (Ti) plasmid, called the transferred-DNA (T-DNA), and integrate it into the host genome causing crown gall tumor.

The *Agrobacterium*-mediated plant transformation method has a number of advantages over other transformation techniques, which include the ability to transfer large intact segments of DNA into a plant cell, thus predominantly generating simple transgene insertions and low-copy-number integration events (Barampuram and Zhang, 2011). At the same time, there are still many economically important crop species and trees that are recalcitrant to *Agrobacterium*-mediated transformation (Păcurar et al., 2011).

Both transient and stable transformation processes are the outcome of the interaction between *Agrobacterium* and its hosts. Hence, two main approaches have been used to elevate the transformation efficiency in already transformable species and increase a number of hosts for *Agrobacterium*-mediated transformation: (i) to identify or engineer highly virulent strains of *Agrobacterium* and (ii) to manipulate host factors involved in the transformation either through the optimization of tissue culture conditions or by directly affecting gene expression in the plant cell (Păcurar et al., 2011). The implementation of the first strategy has resulted in the development of highly virulent strains with a wide range of hosts including both dicots and monocots (e.g., hypervirulent strains carrying the Ti plasmid pTiBo542 and its derivatives) (Cheng et al., 2004; Komari, 1989; Jones et al., 2005; Hood et al., 1987). Nonetheless, enhancing the *Agrobacterium* strains by supplying them with additional copies of *vir* genes has been suggested to reach its limit (Păcurar et al., 2011; Gelvin,

2003). Alternative approaches involving the manipulation of host factors that participate in *Agrobacterium*-mediated plant transformation constitute a promising direction to explore.

The first attempt to identify plant genes involved in *Agrobacterium*-mediated plant transformation utilized a forward genetic screening procedure in 3000 *Arabidopsis thaliana* (*Arabidopsis*) T-DNA insertion mutants to reveal plants recalcitrant to *Agrobacterium* infection (Nam et al., 1999). This study was followed by a larger-scale investigation that involved approximately 16,500 *Arabidopsis* mutants (Zhu et al., 2003). Overall, by using a combination of stable and transient root-based transformation assays, the authors identified more than 120 genes encoding proteins which were required to promote transformation (Gelvin, 2009). It did not come as a surprise that the products of most identified genes were involved in key steps in *Agrobacterium* infection, i.e., bacterial attachment (an arabinogalactan protein), cytoplasmic trafficking of the T-DNA complex (actin-2 and actin-7), nuclear targeting (importin- $\alpha$ 7 and importin- $\beta$ 3) and T-DNA integration/chromatin remodeling (histones H2A, H2B, H3, and H4) (Zhu et al., 2003). At the same time, the authors pointed out that the screen was not saturating, which suggested that the potential for the discovery of new genes involved in *Agrobacterium*-mediated plant transformation was not exhausted. Indeed, the involvement of additional chromatin-related genes (24 genes in total) was further revealed by using RNA interference (RNAi) *Arabidopsis* mutants (Crane and Gelvin, 2007). Hence, it has become apparent that the *Agrobacterium* T-DNA tightly interacts with host chromatin factors, albeit it has yet to be deciphered how particular chromatin proteins affect *Agrobacterium*-mediated transformation at the molecular level.

## 2. Plant epigenetic factors involved in *Agrobacterium*-mediated stable transformation

### 2.1. Roles of histone and chromatin modifications during transformation

After T-DNA enters the nucleus, chromatin proteins may mediate its integration into the genome. For example, histones (H2A, H2B, H3 and H4) can interact with the VirE2-interacting protein (VIP1), a protein which may associate with T-DNA in the cytoplasm and help target it to the nucleus (Magori and Citovsky, 2011). In addition, it was suggested that prior to the integration into the genome, the T-DNA had to interact with host chromatin

**Table 1**  
Epigenetic-related genes that affect plant transformation.

Gene	Gene function	Modulation of gene expression	Effects on plant transformation	Reference
<i>HTA1</i>	Histone H2A-1 involved in nucleosome assembly	Overexpression	A 2-fold increase in the frequency of <i>Agrobacterium</i> -mediated stable transformation of <i>Arabidopsis</i>	Mysore et al. (2000)
<i>HTA1</i>	Histone H2A-1 involved in nucleosome assembly	Overexpression	Up to a 44% and 50% increase in <i>Agrobacterium</i> -mediated stable transformation and the frequency of GT events in rice, respectively	Zheng et al. (2009)
<i>HTA</i> , <i>HTR</i> and <i>HFO</i>	Core histone proteins H2A, H3-11, and H4 involved in nucleosome assembly	Overexpression	A 2-fold increase in the transformation frequency of <i>Arabidopsis</i> root segments	Tenea et al. (2009)
<i>CAF-1</i>	Histone chaperon, involved in nucleosome assembly	Knockout	A 2-fold increase in the T-DNA integration frequency	Endo et al. (2006)
<i>RDR6</i>	Biogenesis of siRNAs derived from posttranscriptionally-silenced transgenes	Down-regulation (RNAi)	An ~1.5-fold increase in the stable transformation frequency	Dunoyer et al. (2006)
<i>DRM1</i> , <i>DRM2</i> , <i>CMT3</i>	The triple mutant that is strongly deficient in CpHpG and CpHpH methylation	Knockout	An ~2-fold increase in the growth of crown gall in <i>Agrobacterium</i> -infected plants	Gohlke et al. (2013)
<i>AGO4</i>	RNA-dependent DNA methylation processes	Knockout	An ~2-fold increase in the growth of crown gall in <i>Agrobacterium</i> -infected plants	Gohlke et al. (2013)

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