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A review on advanced methods in plant gene targeting

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ABSTRACT

Plant genetic engineering is one of the most significant tools implemented in the modern molecular crop breeding techniques. The conventional approaches of plant genetic transformation include *Agrobacterium tumefaciens*, particle bombardment, DNA uptake into protoplast. The transgenic events derived by these methods carry the transgenes that are integrated at random sites in the plant genome. Novel techniques that mediate integration of foreign genes at specific pre-determined locations circumvent many problems associated with the existing methods of gene transfer. The recent years have witnessed the emergence of gene targeting techniques by employing zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindrome repeats (CRISPR). The present review focuses on the various approaches and their performance of plant gene targeting and suggests future directions in the important areas of plant molecular biology.

Contents

1. Introduction

The genetic transformation in plants has revolutionized agriculture by facilitating the introduction of foreign genes into the agronomically and horticulturally important specie. This technology leads to the expression of novel traits such as pest resistance, disease resistance, and quality improvement. The transgenic plants are generated based on the genetic transformation techniques mediated by *Agrobacterium tumefaciens*, particle bombardment, and DNA uptake into protoplast. The transgene integration, mediated by these techniques takes place at random sites in the plant genome. The position of genomic integration and the complexity of the integrated DNA influence the level of transgene expression [58,39,21]. Also, the transgenes inserted at random positions may lead to redundant mutations because of its insertion in the active plant genes [37]. The development of techniques that mediate transfer and integration of the foreign genes at specific predetermined locations obviates many complications associated with the existing gene transfer methods. The introduction of foreign genes via Gene Targeting (GT), which is based on the Homologous Recombination (HR), offers many advantages such as precision gene integration, single copy transgene insertion, and high expression of

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the transgenes. It allows the construction of 'safer' transgenic crops, with no unknown 'position' effects due to random integration.

GT is a genetic technique that uses HR to alter a specific DNA sequence in an endogenous gene at its original locus in the genome. Paszkowski et al. [56] integrated antibiotic-tolerant gene into the tobacco genome using GT originally. Various HR-dependent approaches have also successfully targeted genes in plants [33,75,80].

In this review, we systematically reviewed the performance of various methods and approaches about the introduction of plant gene targeting. The HR, site-specific recombination, Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and clustered regularly interspaced short palindrome repeats (CRISPR) has taken into consideration.

2. Genome editing tools

2.1. Homologous recombination

HR is a DNA maintenance pathway that protects the chromosomes against damages involves both the DNA strands, such as Double Strand Breaks (DSBs) and interstrand crosslinks. HR, illegitimate recombination or Non-Homologous End Joining (NHEJ), and Single-Strand Annealing (SSA) are the three different ways of foreign DNA integration into the native genome. SSA, the third path of repair, requires the presence of repeated sequences on both sides of a break. After the exonuclease degradation of the 5′ ends, repair occurs by annealing of the two complementary sequences, which leads to the loss of the genetic information contained between these repeats.

Puchta [63] reported that one of the most efficient and effective means of improving the frequency of HR frequency to develop a break in the chromosome at the target site. The repair mechanism originates at the break by simulating the cell's DNA system, and the homologous template proceeds through the HR during the repair process. GT has been widely used in mice and yeast, and its efficiency in plants was not sufficient for the routine applications [60,24]. Different methods were tested for increasing the GT efficiency in plants. Moreover, DSBs are created by a rare enzyme, I-SeeI, which could improve the homologous integration frequency at the target site [61]. Such strategy entails transgenic target sites that are randomly inserted into the genome, and thus, it would not be likely to target endogenous genes [62,25,59] referred that *rad9* and *rad17* mutants lead to the induction of high HR frequency.

Several attempts have been made to enhance the competitiveness of the HR machinery by expression of the heterogeneous factors in other organisms. *RecA* DNA recombination protein in *Escherichia coli* has improved the frequency of intrachromosomal recombination [64]. Further, Reiss et al. [64] stated that the line of RecA- overproduction strategy did not modify the GT frequency. Provided the evolutionary conservation of mechanisms in recombination, the presence of complete sequences of Rice and Arabidopsis characterized endogenous regulatory components of HR/NHEJ [50] have applied forward genetic screens for the genotoxic treatments of the affected mutants, indicating the original components that influenced HR levels. The continuation of such efforts would assist in the understanding of the circuits of plant-particular regulation that are included in the repairing of DSB [26].

Previously, the frequency of the HR-dependent GT was identified in the order of 10^{-3} and 10^{-6} with regard to the random combination of GT vector [28,40,79,7,66,23,24,65,32]. Subsequently, it was found that the induction of DSBs maximized the HR frequency by certain magnitudinal orders. Therefore, engineered nucleases were developed as an appropriate method for enhancing the efficiency and successful deployment of GT in mammals that maximized gradually as illustrated by [36]. Voytas [80] stated that DSBs induction at specific genome locations was effective in enhancing the efficiency of GT in plants.

Genome editing, or genome editing with engineered nucleases (GEEN) is a type of genetic engineering to insert, delete or replace DNA in the genome of an organism using engineered nucleases, or "molecular scissors." These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced DSBs are repaired by non homologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations.

There are currently 3 types of engineered nucleases being used: zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the CRISPR-Cas system. Fig. 1 illustrates the structure and mode of action of these nucleases and Table 1 shows the comparison between these technologies.

2.2. Zinc finger nucleases

ZFNs are restriction enzymes with Zinc Finger (ZF) domains that recognize a particular sequence of DNA, fused to the nuclease domain of restriction enzyme *Fok*I [38]. Since the domain of ZF could be engineered to focus on novel sequences of DNA, ZFNs were exploited to engineer the endogeneous genome loci, particularly in the eukaryotic systems, [10]. According to [45], in the case of ZFNs, one module of DNA-binding involves nearly 30 amino acids and identifies 3 nucleotides integrating module of DNA-binding. It allows the recognition of 9–18 bps of DNA sequences. ZFNs were used for creating breaks in the site-specific chromosome, particularly in the absence of pre-engineered sites for the target [3,76].

The development of ZFN-mediated GT provides Molecular Biologists with the ability to modify the plant genomes site-specific and permanent via homology-directed repair of a targeted genomic DSB. ZFNs can be used to induce DSBs in specific DNA sequences and thereby promote site-specific HR and targeted genomic manipulation. ZFNs have a DNA recognition domain that involves an array of Cys_2-His_2 ZF. ZFs recognize and bind to particular nucleotide triplets. Various ZFs can be combined together for generating DNA-binding arrays that would identify the expanded sequence patterns with high affinity and greater specificity [15,55], and [68]. The gene constructs were made from the custom-designed ZFNs which were designed to cut at specific DNA sequences at a preselected locus in the plant genome. This was due to the efficiency and directiveness of the ZFs for a broad range of DNA sequences. A site-specific ZF endonuclease has been successfully employed to induce site-specific mutations by Non-Homologous End Joining in Arabidopsis [46].

By convention, the targeted genome modification (TGM) was frequently performed using the synthetic domain of ZFNs that is fused to a cleavage domain of Fok1 [77,11]. ZFNs were used to modify endogenous genes in various organisms, cell types, and plant species including Arabidopsis [54,82], soybean [18], maize [70] and tobacco [74]. Most of the constraints on the application of ZFN encompass the limited quantity of existing target sites, more effects on context dependence between the low targeting specificity and efficiency, repeat units and the effects of frequent off-target caused partially by the non-specific binding of DNA [20].

In Drosophila, the engineered ZFNs have identified the yellow gene that was observed in the larvae in the presence of the donor DNA. It was either joined elsewhere in Drosophila genome or seen as free-floating molecules released from the chromosomes of Drosophila by FLP recombinase [3].

Bozas et al. [6] studied the genetic analysis of ZFNs-induced GT in Drosophila. Using ZFNs for cleaving the target in the

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