Chapter 35
Genome Editing

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ABSTRACT

Targeted editing of the genomes of living organisms not only permits investigations into the understanding of the fundamental basis of biological systems but also allows to improve productively and quality of crops. This includes the creation of plants with valuable compositional properties and with traits that confer resistance to various biotic and abiotic stresses. Recently, several novel genome editing systems have been developed, which include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALNEs), and clustered regularly interspersed short palindromic repeats/Cas9 (CRISPER/Cas9). These exciting new methods have proved themselves as effective and reliable tools for the genetic improvement of plants. The genome editing systems can also be used to exploit the genetic diversity present in the semi-domesticated and wild relatives of the cultivated crops by targeting homologous domesticated genes through allele-mining. In this chapter various tools available for gene editing, their merits, and demerits have been discussed.

INTRODUCTION

Genome editing may be defined as a set of advanced molecular biological techniques that facilitates precise, efficient, and targeted modifications at specific loci in a genome. Use of transcription activator such as effector nucleases (TALENs), and zinc-finger nucleases (ZNFs) has been in use for such purposes, but came to limelight recently due to discovery of CRISPER (Clustered Regularly Interspersed Short Palindromic Repeats) system, which is comparatively simple and easy to use for gene editing. The gene editing technologies basically use sequence-specific nucleases (SSNs) that can induce to identify specific DNA sequences and create double-stranded breaks (DSBs). Organisms internal repair systems fixes the DSBs either by homologous recombination (HR) or non-homologous end joining (NHEJ) mechanisms. The HR can cause gene replacements or insertions, while NHEJ can cause insertions or deletions of nucleotides, which leads to gene knockouts. Since gene editing technologies alter target design genetically they are considered to be different from genetic engineering technologies used for development of

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genetically modified (GM) crops. After completion of editing the gene, it is not possible to distinguish between the edited gene and the naturally occurring mutants, in the segregating generations. Therefore, application of gene editing system should enhance the crop improvement process.

ZINC-FINGER NUCLEASES

Zinc finger nucleases (Z NFs) is considered to be the first generation genome editing tools that was developed through chemically engineered nucleases, having folded up to ββα configuration. The enzyme is derived from fusion of zinc-finger based DNA-recognition modules and the DNA-cleavage domain of the *Fok*I restriction endonucleases (Figure 1a). The α-helix of the protein gets inserted to the DNA after binding of the protein into the major groove of the double helix of DNA. Each zinc finger recognizes and binds to a triplet nucleotide sequence and assembles into a group at the specific binding site(s). The monomer of ZFN has two different functional domains: an artificial ZF Cys2-His2 domain (N-terminal end) and a non-specific *Fok*I DNA cleavage domain (C-terminal end). Dimerization of the *Fol*I domain is critical for enzymatic activity of ZEN. The individual zinc finger domains are interchangeable and according to the order of the domain, they can bind to specific sites sequences in the genome. Several zinc finger domains capable of recognizing large numbers of triplet nucleotides has been generated with the objectives to target large sequences of interest.

ZFNs have so far been used to modify rice, maize, soybean, rapeseed, tobacco, apple, fig petunia, and *Arabdiopsis*. Compared to other tools, ZFN has been found to been efficient, high specificity, and minimal non-target effects. Although ZNFs have been used successfully for development of herbicide tolerance and stacking useful traits in maize and identification of safe regions for gene integration in rice, the technique has remained complicated and technically challenging for crop improvement. Therefore, the current focus is on to improve the design and delivery system of ZNF technology for wider acceptability for crop improvement.

TRANSCRIPTION ACTIVATOR-LIKE EFFECTOR NUCLEASES

Transcription activator-like effector nucleases (TALENs) are a class of enzymes derived from the transcriptional activator-like effectors (TALE) repeats and the *Fok*I restriction enzyme (Figure 1a). The central domain of TALE protein is responsible for binding of DNA and nuclear location signal. It also serves as the activator of transcription of the target gene. In the TALE monomer, the DNA-binding domain is consist of a central repeat domain (CRD) which is responsible for binding of DNA and host specificity. The CRD is made up of 34 amino acid residues that are arranged in tandem repeats. Each of the 34 amino acid long repeats binds to one nucleotide in the nucleotide sequence of the target gene. In the 34 amino acid repeats, two amino acids (located in 12 and 13 positions) are highly variable (called, repeat variable diresidue, RVD) and are responsible for the recognition of specific nucleotide. The last tandem repeat which binds to the nucleotide at the 3'-end of the recognition site has only 20 amino acid residues, and thus it is called half repeat. Although, TALE protein can be designated to bind any DNA sequence of interest, it is interesting to note that 5'-most nucleotide base of the DNA sequence should be thymidine, where TALE protein binds. Any deviation from this requirement can affect the efficacy of TALE recombinase (TALE-R), TALE transcription factor (TALE-TF) and transcription activator-