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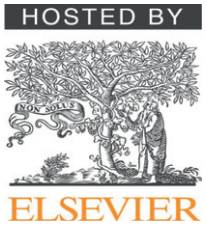

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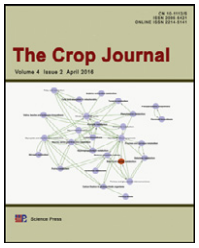

CRISPR/Cas9: A powerful tool for crop genome editing

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ABSTRACT

The CRISPR/Cas9 technology is evolved from a type II bacterial immune system and represents a new generation of targeted genome editing technology that can be applied to nearly all organisms. Site-specific modification is achieved by a single guide RNA (usually about 20 nucleotides) that is complementary to a target gene or locus and is anchored by a protospacer-adjacent motif. Cas9 nuclease then cleaves the targeted DNA to generate double-strand breaks (DSBs), which are subsequently repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms. NHEJ may introduce indels that cause frame shift mutations and hence the disruption of gene functions. When combined with double or multiplex guide RNA design, NHEJ may also introduce targeted chromosome deletions, whereas HDR can be engineered for target gene correction, gene replacement, and gene knock-in. In this review, we briefly survey the history of the CRISPR/Cas9 system invention and its genome-editing mechanism. We also describe the most recent innovation of the CRISPR/Cas9 technology, particularly the broad applications of modified Cas9 variants, and discuss the potential of this system for targeted genome editing and modification for crop improvement.

Contents

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1. Introduction

Since the inception of genetic engineering, methodologies for modifying a specific genetic locus of a target organism with a single-base resolution have been eagerly pursued. The invention of CRISPR/Cas9 technology has made this dream come true and opens a new era for genome editing. The technique is extremely simple, economical, and versatile in many applications with minor modifications. CRISPR/Cas9 is commonly used in mammals and plants, for both basic scientific research and genetic engineering. The technique is rapidly evolving and its application is constantly expanding. In this review, we describe how CRISPR/Cas9 works and how it can be applied in plants, especially crop plants. We also discuss the pitfalls of this technique and its future development for crop genetic improvement.

2. The CRISPR/Cas9 system: from bacterial immunity to genome editing

CRISPR is an acronym for clustered regularly interspaced short palindromic repeats and Cas9 is a nuclease associated with CRISPRs. These 29-nucleotide (nt) repeat sequences separated by various 32-nt spacer sequences were first reported in bacteria as early as 1987 [1]. Later, they were found in 40% of sequenced bacterial genomes and 90% of archaea [2]. Meanwhile, several types of Cas genes were found to be well conserved and adjacent to repeat elements [3]. These CRISPR/Cas systems can be classified into types I, II, and III, with the type II system requiring only the Cas9 nuclease to degrade DNA that matches a single guide RNA (sgRNA) [2]. The year 2005 was remarkable in the CRISPR/Cas9 epoch; in that year the spacer sequences were found to be originated from phage genomes [4–6]. Based on this discovery and the findings that viruses are unable to infect archaeal cells carrying sequences matching their own genomes, CRISPR/Cas systems were hypothesized to serve as a critical immune system to protect owners from pathogen invasion [5]. By 2011, the mechanism by which Cas9 works with CRISPR RNA (crRNA) and trans-activator crRNA (tracrRNA) to attack foreign DNA that matches the crRNA was decoded [7]. Soon, the tracrRNA and crRNA were combined into a single guide RNA molecule, an advance that has since rapidly accelerated the application of the CRISPR/Cas9 system in practice (Fig. 1-A) [8].

3. The Cas9 nuclease: the structure and the working mechanism

Unlike random mutagenesis, such as EMS mutagenesis and radiation [9], targeted genome-editing provides precise and

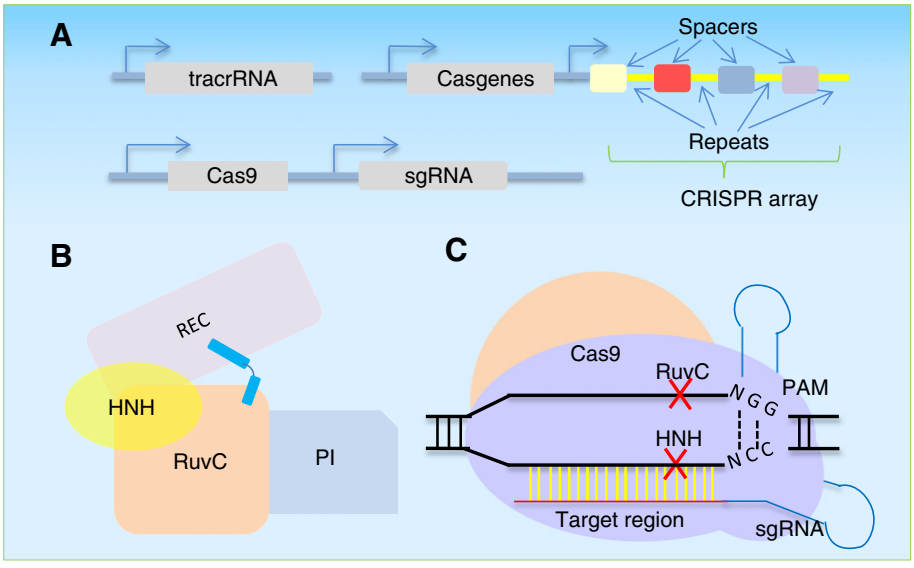

Fig. 1 – Components of the type II CRISPR/Cas system. A. Genomic structures of the native bacterial CRISPR/Cas system (top) and the engineered CRISPR/Cas9 system (bottom). tracrRNA, trans-activator RNA; sgRNA, single guide RNA; B. A schematic representation of the Cas9 protein structure. Domains include REC (large recognition lobe) and RuvC (a nuclease domain) which is linked with an arginine-rich region. HNH is a second nuclease domain. PI, PAM-interacting domain. C. The conformation of Cas9-sgRNA complex in the process of DNA cleavage.