biology and life sciences forum

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Proceeding Paper

Clustered Regularly Interspaced Short Palindromic Repeats-Cas: A Potential Genome Editing Tool in Crop Improvement †

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† Presented at the 3rd International Electronic Conference on Agronomy, 15–30 October 2023; Available online: https://iecag2023.sciforum.net/.

Abstract: An innovative method, the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing system, has significantly revolutionized agriculture by improving the quality of crops and sustaining the environment. CRISPR technology is based on the natural defense mechanism that bacteria and archaea have adapted against invading viruses or other foreign DNA. A genome engineering tool employs a similar mechanism for exceptional crop breeding progression due to its precise gene editing accuracy. This study outlines the present application of CRISPR/Cas9 technology to assess agricultural crop yield, quality, and texture modulation, palatability, nutritional components, disease resistance, and environmental stress. In plants, CRISPR/Cas9 geneediting includes the selection of specific target sites, single guide (sgRNA) design and synthesis, ribonucleoprotein (RNP) or transformation carrier delivery in plant cells, and gene-edited plant transformation and regeneration. The knockout of three mlo genes in wheat confers wheat resistance to powdery mildew disease. The CRISPR/Cas9 system knockout gene Clpsk1, which encodes phytosulfokine, indicates that watermelon with enhanced *Fusarium* wilt disease resistance can regulate plant immunity. The geneppa6 knockout has improved rice's ability to withstand alkaline stress. Furthermore, the simultaneous editing of multiple genes has contributed to pathway-level plant biotechnology research that widely expands the genome engineering of agronomic traits and its adoptability. All the CRISPR/Cas systems require a specific PAM sequence, which guides the editing sites with specificity. Consequently, developing a PAM-independent CRISPR/Cas system, exploring new relationship between Cas proteins and the modification of Cas enzymes for expanding PAM variants will boost the application of CRISPR/Cas in applied research on agriculture, precision breeding, and ensuring food security.

Keywords: genome editing; CRISPR; Cas9; agriculture; crop improvement

Citation: Mamatha Bhanu, L.S. Clustered Regularly Interspaced Short Palindromic Repeats-Cas: A Potential Genome Editing Tool in Crop Improvement. *Biol. Life Sci. Forum* **2023**, *27*, 48. https://doi.org/10.3390/IECAG2023-15873

Academic Editor: Gianni Bellocchi

Published: 7 November 2023

1. Introduction

The agricultural food production system is currently facing challenges due to climate change and environmental stress, resulting in reduced grain quality and crop yield. Crop yield and quality areof utmost significance to provide nutritional security to mankind; the current scenario of food security is challenging with the growing population and extreme climatic fluctuations [1]. By 2025, the global population is projected to reach nearly 10 million, necessitating urgent efforts to eradicate global hunger., a sustainable increase in food production by around 60–100% is needed (FAOSTAT, 2016). World food production and its distribution depends on farmers, breeders as well as policy makers and the government adapting scientific approaches to ensure food security and eliminate hunger [2]. Traditional breeding methods are insufficient for growing populations, leading to the use of recent genome editing techniques for effective population growth management. Genome editing (GE) is a heritable technique that involves the deletion and insertion of single nucleotides

or large-fragment substitutions in the plant genome [3]. However, Genome editing significantly impacts the agronomic quality traits of various monocots and dicots, thereby reducing environmental stress, climatic fluctuations, yield, and nutritional quality [4]. Genome editing involvessequence-specific nucleases that targets the DNA at a specific site and create double-stranded breaks (DSB); these breaks are repaired through (NHEJ) non-homologous end joining or (HDR) homologous-directed recombination pathways, producing insertion, deletions (INDEL) or substitutions of the base in the target region of the DNA [5]. Gene-edited crops are of much use nowadays for breeding new varieties, as there areno marketing and consumption issues. Waltz [6] argues that the ethical issue surrounding genetically modified crops is less significant than that of genome-edited crops. First-generation genome editing technologies, developed in the 1900s, involve zinc finger nucleases (ZFNs) and FoK1 endonuclease breaks on DNA zinc finger motifs [7]. This has been advantageous in many plants, like maize, soya bean and tobacco [8]. Transcription activator-like effector nucleases (TALENs), a substitute for ZFNs, are naturally occurring extended segments of transcription activator-like effector (TALE) sequences attached to the Fokl domain, with TALE repeat arrays [9]. They are advantages over ZFNs and areused to initiate non-homologous mutations in plants [10] and are used in rice [11], tobacco [12] and Arabidopsis [13]. The advancement of genome editing technologies, particularly CRISPR/Cas9, has significantly influenced plant breeding research, enabling effective application of this second-generation gene editing technique in various crop plants [14].

2. CRISPR/Cas9 GeneEditing Technology

Escherichia coli were the first model organism to be studied using the CRISPR system, and archaea were also examined over 30 years ago [15,16].Cas proteins, linked to CRISPR, are involved in DNA repair, forming an adaptive immune system RNA-guided and regulated by CRISPR RNA (crRNA) with either class 1 or class 2 Cas proteins. [17,18] Based on a particular protein that cleaves specific DNA, the two classes of Cas proteins are divided into three types. In the class 1 CRISPR-Cas systems, the effector module consists of a multi-protein complex in the effector module with three types, I, III and IV, whereas the class 2 systems have a single effector protein with the II, V and VI types [18]. Furthermore, based on the CRISPR-Cas locus architecture, there are many subtypes. Makarova et al. (2020) reported two classes of CRISPR-Cas, six types and thirty-three subtypes.

MechanismsofCRISPR/Cas9

CRISPR-Cas9 recognizes and cleaves foreign DNA or RNA segment in a sequence-specific manner. This is an adaptive defense mechanism in prokaryotes which can be divided into three stages: (i) spacer acquisition/adaptation, (ii) the biogenesis of crRNA, and (iii) target interference [19]. In The CRISPR array includes a sequence of mobile genetic elements and a protospacer for spacer acquisition/adaptation, resulting in the creation of a new spacer. This process allows one to memorize the foreign DNA/RNA inthe host organism, and then it is transcribed into long-precursor CRISPR RNA (pre-crRNA) by the two proteins, Cas1 and Cas2 [20]. The spacer acquisition event is processed by the Cas6 protein in the type I and III systems.In the type II CRISPR-Cas systems, crRNA maturation requires tracrRNA, RNase III and the Cas9 protein. In the type II-A CRISPR-Cas systems, the (protospacer adjacent motif) PAM-recognizing domain of Cas9 is involved in protospacer selection [21]. Later, Cas9 recruits the other proteins, Cas1, Cas2 and possibly Csn2, for the integration of the new spacer into the CRISPR array, which is conserved among all the class II CRISPR-Cas systems [22]. The biogenesis of crRNA after adaptation is a crucial process, the CRISPR array is transcribed into a long-precursor crRNA (pre-crRNA) that is again processed into mature guide crRNAs containing the memorized foreign sequences [23]. The type I and III Cas6 proteins carryout the processing step to obtain intermediate species of crRNAs that are flanked by a short 5′ tag. In the type II systems, tracrRNA carries out the processing of pre-crRNA. The anti-repeat sequence of this RNA creates an RNA duplex that repeats the pre-crRNA using Cas9. The duplex is cleaved

by RNase III, forming an intermediate crRNA that undergoes further maturation, resulting in the mature small-guide RNA. Small-guide RNA, which has matured guide interference with invading nucleic acids, serves as the final defense step against these invaders [24]. The class 1 systems engage cascade (CRISPR-associated complex for antiviral defense)-like Cas3 complexes to achieve target degradation, while in the class 2 systems, a single effector protein is sufficient for target interference; the tracrRNA: crRNA duplex guides the effector protein Cas9 to create a break in the target double-stranded DNA [5,25,26].

3. Applications of CRISPR-Cas in Crop Improvement

CRISPR/Cas9 gene editing has been successfully utilized to develop disease-resistant crop varieties and enhance their resistance to significant environmental stressors. Shan et al. [27] study tested rice protoplasts for rice genes responsible for abiotic stresses using CRISPR-Cas9 technique, including betaine aldehyde dehydrogenase (OsBADH2), mitogen-activated protein kinase (OsMPK2) and phytoene desaturase (OsPDS). The CRISPR/Cas9-mediated genome editing of OsERF922 in rice has been found to enhance resistance against the pathogen *Magnaporthe oryzae*, which causes blast disease [28]. Gene editing wasalso demonstrated in wheat; CRISPR TaMLO knockout showed resistance to powdery mildew disease caused by *Blumeriagraminis* f. sp. *Tritici* (Btg). Kim et al. [29] successfully expressed around 70% of wheat protoplasts for dehydration-responsive element binding protein 2 (TaDREB2) and wheat ethylene-responsive factor 3 (TaERF3), revealing the importance of these proteins in the plant's development. Similarly, the genes ZmIPK1A, ZmIPK, and ZmMRP4 were targeted to knockout, resulting in the synthesis of anti-nutritional factors, such as phytic acid [30]. Furthermore, the maize U6 snRNA promoter modified the carotenoid biosynthesis gene (PSY1) in maize, resulting in white kernels and albino seedlings [31]. The studies on Arabidopsis by Wang et al. [32] showed CRISPR/Cas9-based target genome editing;three phenology-related *Arabidopsis* genes, brassinosteroid insensitive1 (BRI1), jasmonate-zim-domain protein 1 (JAZ1) and gibberellic acid insensitive (GAI), were observed in the succeeding generations. A CRISPR/Cas9 genetic improvement study on the *Wx* gene in the japonica rice variety successfully produced 5–12% grain amylose content. The knockout of the gene *DcMYB7*, *R2R3-MYB*, in the solid purple carrot resulted in yellow roots [33]. Most of the World Trade Organization members are supporting the use of gene editing in agricultural innovation; this isthe first step towards establishing a worldwide regulatory framework for a hunger-free world [34].

4. Conclusions

CRISPR/Cas-mediated gene editing is a game-changing technique with wide application in crop improvement to increase the yield, nutritional value, disease resistance and tolerance to environmental stress. In the last decade, it has beenused in many plant systems, both in dicots and monocots, to combat abiotic and biotic stresses and to improve the desirable agronomic traits. However, CRISPR/Cas9-based genome editing is gaining popularity with several modifications to obtain suitable, edited, desired plants that will help achieve the zero hunger sustainable goals ofthe growing human population.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

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