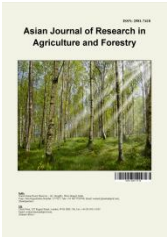

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Role of CRISPR in Crop Improvement: A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors contributed to the study's conception and design. Author HR was responsible for creating the study and writing the protocol. Authors HR and MAA handled the preparation of the materials, data collection and analysis. Author HR wrote the first draft of the manuscript, and author SK provided feedback on earlier iterations. Authors MHS, ME and MRK the literature searches and contributed a lot to strategies portion. The final part of the manuscript is hinder hunger written by authors AS and MHS. Author HR was in charge of managing the references and citations. All authors read and approved the final manuscript.

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ABSTRACT

The meals and agriculture sectors have witnessed good sized advancements due to the state-of-the-art improvements in agricultural biotechnology and genetic engineering, that have more advantageous the essential characteristics of plant agronomic tendencies. A extensively used

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technique for inducing focused deletions, insertions, and precise sequence adjustments throughout numerous species and mobile kinds is collection-particular nucleases (SSNs)-based centered genome editing. Commercial adoption of genome modifying gear, which include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and siRNA-mediated RNA interference, has been significant. However, the whole landscape of lifestyles sciences underwent a paradigm shift with the invention of the CRISPR/Cas9 machine as a flexible tool for genome modifying. Initially recognized as a virological defense DNA segment in bacteria and archaea, the clustered Regularly interspaced short palindromic repeats (CRISPR) machine has revolutionized molecular biology. Through modern molecular organic strategies, CRISPR/Cas9 enables unique modifications in any crop species. Its efficacy, reproducibility, and specificity have earned CRISPR/Cas9 the moniker of a "leap forward" in biotechnology.

Keywords: Genome editing; CRISPR/Cas; crop improvement; gene function; palindromic; commercial adoption; crop species.

1. INTRODUCTION

Developing desired agronomic trends in crop vegetation is difficult due to their complicated genome organisation and gene expression systems. Site-precise mutagenesis, a not unusual method for trait improvement, may be hard or maybe impossible [1]. Consequently, oblique technique like RNA interference (RNAi) are regularly hired to silence goal genes. However, RNAi-mediated gene disruption can also sometimes bring about choppy or partial effects. The introduction of genome editing, additionally referred to as targeted genome editing (TGE), genome editing with engineered nucleases (GEEN), or artificially engineered nucleases (known as "molecular scissors"), has transformed the ability to carry out targeted changes in a wide range of crop species crucial to agronomy. Successful genome modifying is predicated on two herbal DNA restore mechanisms: non-homologous quit becoming a member of and homology-directed repair. Various nucleases, such as Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), Mega-nucleases, and the CRISPR/Cas machine, have been advanced for precise genome editing. In assessment to ZFNs, TALENs, and mega-nucleases, the CRISPR/Cas9 gadget recognizes goal web sites via a complementary sequence-based interplay among the target website online's guide RNA and DNA [2]. The complicated fashioned with the aid of the guide RNA and Cas protein possesses the essential nuclease pastime for specific cleavage of double-stranded DNA the use of the Cas9 endonuclease. This revolutionary era lets in for extra correct and efficient genome modifying as compared to standard strategies like RNA interference. Additionally, ZFNs and TALENs are more expensive genome editing tools because they need protein engineering before they can be used. In contrast, CRISPR/Cas9 is the ideal genome editing tool because it is much less expensive, has a high efficiency in editing the target genome, and is widely used to edit the genomes of plants to create novel genotypes with desired agronomic traits that will strengthen global food security. Since the domestication of crops, researchers and breeders have worked to enhance various characteristics of crops to improve their quality, yield, and growth as well as their ability to withstand a variety of abiotic stresses like salinity and drought as well as pests and other environmental factors. In the beginning, people just chose the morally upright individuals from the expanding field, even if they had no idea why there was a difference [3]. Prior to Gregor Mendel's discovery of the basic principles of heredity in the 1800s, people were beginning to understand that genes regulated every agricultural attribute and that the phenotypic of a single plant is determined by the mix of genes from both parents. Conventional breeding, followed by molecular and transgenic breeding, is based on Mendel's Law of Inheritance. Breeders have been combining desirable qualities via backcross and cross breeding for a long time. Therefore, more precise gene editing techniques are needed for both crop enhancement and gene function research. As of right now, four main families of nucleases have been identified for use in genome editing: zinc finger nucleases (ZFNs), meganucleases, transcription activator-like effector-based nucleases (TALEN), and clustered regularly interspaced short palindromic repeats associated nucleases (CRISPR/Cas) [4]. As also of these systems need a lab and are expensive, however, and they are also challenging to operate. Subsequently, scientists were interested in the

CRISPR/Cas system, which has since gained popularity as a genome/gene editing technique.

A broad class of short palindromic repeat sequences known as CRISPR is found in many prokaryotes, such as the majority of bacteria and archaea. These brief repetitive sequences are complementary to certain foreign DNA sequences in prokaryotes, including viral DNA that infiltrates bacteria or Archaea [5]. This form of DNA is produced by bacteria when they are infected by viruses, and it binds to the DNA of the virus. The Cas enzyme then utilizes this invading DNA to break it apart. As a result, prokaryotes use CRISPR/Cas as a kind of acquired immunological resistance against viruses. It is a naturally occurring tool for genome editing as well. In 1987, *Escherichia coli* was accidentally used to clone the first CRISPR. Subsequently, additional bacteria and Archaea were also cloned with comparable CRISPR sequences. But for the first almost ten years, scientists were unaware of the purpose of these unique repetitive sequences and just believed they were unique sequences found in other species, which is how they were employed for strain typing [6]. The halophilic Archaea *Haloferax volcanii* was created in 1995 by Mojica and colleagues using a plasmid carrying a fragment of CRISPR sequences; their findings showed that the addition of more copies of CRISPR sequences changed the genome's distribution. This is the first instance of foreign plasmid CRISPR and Archaea being incompatible. Nevertheless, until the discovery of CRISPR-associated protein (Cas) genes and their roles in bacterial defense systems, their true purpose remained unknown [7]. This lays the groundwork for the genome editing tool CRISPR/Cas. Their research, along with previous studies, demonstrates that the Cas enzyme, crRNA, and tracRNA are the three components needed for the CRISPR/Cas system to operate when it comes to genome editing. Single guide RNA (sgRNA) is a chimeric synthetic RNA molecule that may be created by combining CRISPR RNA (crRNA) with trans-activating crRNA (tracRNA). SgRNA is ideal for genome editing activities [8]. Additionally, used Cas9 to eliminate functional genes in many human cell lines. Both experiments used a single crRNA-tracRNA fusion sgRNA transcript to modify the Cas9 enzyme, enabling its high function in both human and mouse cells. Additionally, both investigations demonstrated the versatility of multiplex editing by introducing numerous gRNAs to target many genes at once [9]. The scientific world swiftly embraced the CRISPR/Cas system to modify, control, and/or observe genes in bacteria, plants, and animals as a result of these two investigations [10].

2. HISTORICALLY, GENE TRANSFORMATION MEDIATED BY AGROBACTERIUM

Agrobacterium-mediated gene transformation stays the primary approach for introducing Cas genes and gRNAs into plant cells, akin to standard transgenic strategies. In this technique, the Cas gene and gRNAs are included into the Ti plasmid using specialised promoters [11]. Typically, the CaMV 35S promoter is utilized to force Cas enzyme expression, at the same time as the U6 promoter is employed for gRNA expression, making sure robust expression of each Cas proteins and gRNAs within centered cells. However, alternative promoters are also utilized for expressing Cas proteins and/or gRNAs [12]. Promoters derived from small RNA genes, which includes U6, are normally selected for gRNA expression because of their compact size. Conversely, promoters remoted from protein-coding genes, like CaMV 35S, are frequently preferred for driving Cas protein expression, aiming for excessive expression degrees.

Although many studies employ promoters from version plant species like *Arabidopsis* for greater gRNA production and genome editing performance, there is developing proof suggesting that cloning promoters from the target plant species may provide blessings [13]. The primary hurdle in CRISPR/Cas-primarily based genome editing lies in plant transformation and the following regeneration of altered cells into whole plants. This undertaking seriously limits its software across all plant species. Without set up methods for plant tissue way of life and regeneration, changing genes in unique plant species becomes distinctly hard, regardless of one's proficiency in genome enhancing [14]. Some modern in planta transformation techniques were explored to triumph over this obstacle. In a recent have a look at, genome modifying events have been caused de novo with the aid of introducing the CRISPR/Cas9 machine into tobacco flora along developmental regulators. Specifically, developmental regulators like WUSCHEL.

3. CRISPR SYSTEM IN NATURE

By looking for CRISPR spacers, viruses in natural habitats may be linked to their bacterial or archaeal hosts. Three steps comprise adaptive immunity: (i) insertion of a brief invasive DNA sequence into the CRISPR array as a spacer sequence; (ii) transcription of precursor crRNA (precrRNA), which matures to produce individual crRNAs, each of which consists of an invader targeting spacer portion and a repeat portion; and (iii) crRNA-directed cleavage of foreign nucleic acid by Cas proteins at sites complementary to the crRNA spacer sequence [15].

4. CREATION OF CROP PLANTS IMMUNE TO ABIOTIC STRESS

Dry farming methods are mostly used to grow maize, and drought tolerance in maize is a significant problem. Under stress conditions related to flowering and grain-filling, ARGOS8 mutants showed increased grain production in comparison to the wild-type. Herbicide resistance in maize was elevated by a CRISPRCas9-mediated mutation that targeted ALS1 and ALS2. Chlorsulfuron resistance in maize might be effectively provided by ALS2 gene editing with single-stranded oligonucleotides serving as repair templates [16].

5. INCREASING THE CROP PRODUCTION LEVEL BY ENHANCING THE CROP'S NUTRITIONAL VALUE

5.1 Gene Regulation by CRISPR/Cas

Utilizing CRISPR-Cas9 gene editing technology, researchers focused the amendment of resistant starch and amylose stages in grains, mainly rice. By using the CRISPR-Cas9 approach, specific mutagenesis changed into caused within the rice SBEI and SBEIIb genes, which are vital for starch biosynthesis. This centered method caused considerable changes in the composition of rice grains. The resulting rice mutants exhibited a wonderful boom in each amylose and resistant starch contents, with levels growing by 25% and 8%, respectively, compared to the wild-type opposite numbers. These upgrades in amylose and resistant starch content contribute to stepped forward nutritional characteristics of the rice grain, presenting potential health advantages to clients [17].

This successful software of CRISPR-Cas9 generation demonstrates its efficacy in exactly modulating starch composition in rice grains, paving the manner for the development of novel rice sorts with more advantageous dietary profiles and probably improved health effects for consumers. Pioneering the use of CRISPR/Cas technology for crop development is the seed business Corteva Agriscience, which was formed via the merging of Dow, Dupont, and Pioneer. The first commercial crop using this technique was created by the company's experts in the spring of 2016: a new variety of waxy maize. The use of CRISPR-Cas9 technology is critical for producing potatoes faster and with larger yields [18]. CRISPR-Cas9 was temporarily expressed in order to achieve gene deletion in tetraploid potatoes (*Solanum tuberosum*). Without integrating DNA, RNP-delivery of the CRISPR-Cas9 machinery produced commercial lines with greater yields [19].

6. UTILISING CRISPR/CAS TO ENHANCE AGRICULTURAL YIELD

Enhancing agricultural output through traditional breeding and transgenic technologies remains an extended-term objective. However, development in utilising gene cloning and transgenic strategies has been limited, more often than not due to the complexity of yield characteristics, which are managed with the aid of more than one genes and gene networks [20]. Despite the mission of figuring out a single gene that governs yield, research shows that the expression of particular genes could have destructive consequences on agricultural output. Consequently, there is growing interest in inhibiting or getting rid of those genes to potentially growth crop yields. The swiftly advancing CRISPR/Cas device gives a great tool for concentrated on and eliminating genes that negatively impact plant productiveness [21]. Studies have verified sizeable improvements in diverse components of crop manufacturing, consisting of large grain length, better grain weight, elevated grain quantity, and denser panicles, thru the knockout of genes like gn1a, dep1, gs3, gw2, gw5, tgw6, vin2, and GASR7 in rice and wheat. Additionally, modifying cis-regulatory elements like CLV-WUS has resulted in large fruits in tomatoes [22].

Field exams have further confirmed the potential of CRISPR/Cas-mediated genome editing to reinforce agricultural productivity. For instance, transgenic maize with the AGROS8 gene edited by means of CRISPR/Cas exhibited a sizable

boom in grain yield, especially below drought situations [23]. Similarly, CRISPR/Cas9-edited versions of OsLOGL5 in rice constantly improved grain manufacturing across extraordinary environmental situations, consisting of drought and coffee nitrogen remedies. The knockout of G protein genes along with gs3 and dep1 in rice has led to improvements in agronomic developments like grain length and amount in line with panicle, in addition highlighting the effect.

7. ENHANCES PLANT RESISTANCE TO PATHOGEN AND INSECT INFESTATION USING CRISPR/CAS GENOME EDITING

Biotic variables have an impact on plant growth and development as well as crop output and quality, sometimes even leading to a total loss of yield. The method and process by which plants react to different diseases complex, and several genes are involved in it [24]. Little progress has been achieved since no one gene significantly dominates the way in which plants respond to diseases despite the fact that transgenic technology has been used to overexpress certain genes in order to create transgenic plants with great resistance to various pathogens. But it seems that deleting a bad gene makes plants more resistant to certain infections [25].

8. CRIPSR/CAS GENOME EDITING ENHANCES THE DEVELOPMENT AND GROWTH OF PLANTS

For instance, the enzyme arginase (ARG) performs an important position in regulating root growth with the aid of inhibiting nitric oxide synthase (NOS). Through CRISPR/Cas9 technology, the ARG gene changed into successfully removed in cotton, main to suppression of lateral root improvement. The knockout lines of ARG exhibited a giant boom in lateral root wide variety (25%) and total root floor region (52%) throughout special nitrogen mediums. This enhancement in root improvement is expected to improve water and nutrient absorption from the soil, thereby selling usual plant growth and development, and enhancing the plant's capability to adapt to various environmental situations [26].

In rice, the significance of MADS container transcription aspect genes MADS78 and MADS79 in endosperm cellularization and early seed development become elucidated the use of CRISPR/Cas9-primarily based genome editing. Double mutants of MADS78 or MADS79 resulted in impaired seed development and lack of ability to supply feasible seeds, while unmarried knockout mutants exhibited premature endosperm cellularization [27]. Additionally, mutations generated through CRISPR/Cas9 inside the hexokinase hxk5 gene brought about male sterility in rice, much like the consequences determined in *Arabidopsis* with sarib and saric gene knockouts of COPII additives affecting pollen formation. Furthermore, in allotetraploid oilseed rape, mutants of the Bnspl3 gene created using CRISPR/Cas9 generation displayed developmental delays. Similarly, CRISPR/Cas9 deletion of the terminal flower1 (tfl1) gene in *Brassica napus* altered segment transition and flowering time [28].

9. CROP QUALITY AND SECONDARY METABOLISM ARE ENHANCED BY CRIPSR/CAS GENOME EDITING

CRISPR/Cas technology has opened avenues for altering flowers' secondary metabolism to beautify crop best. The composition of practical additives which include proteins, carbohydrates, oil, and bioactive substances performs a pivotal position in determining agricultural product first-rate [29]. By leveraging CRISPR/Cas-mediated multiplex genome editing, researchers have centered specific genes inside the carotenoid metabolic pathway to enhance lycopene biosynthesis and manufacturing in tomato end result. Lycopene, a famous practical bioactive ingredient, holds promise in treating various human illnesses, consisting of cancer and cardiovascular issues. Protein nice is some other critical element of agricultural products, with gluten sensitivity affecting about 1% to 2% of the populace. CRISPR/Cas9 genome editing technology has been applied to broaden low-gluten transgene-unfastened wheat by using concentrated on specific α-gliadin genes. Additionally, alterations in protein profiles and amino acid composition were performed in Camelina sativa seeds thru CRISPR/Cas9-mediated modifying of the CRUCIFERIN C (CRUC) gene.

Deletion of pat2/5 genes has caused multiplied starch accumulation in rapeseed seeds, even as knockout of starch branching enzyme (SBE) genes has ended in rice with excessive amylose content. Similarly, genome editing of granule-certain starch synthase (GBSS) genes in

potatoes has motivated starch satisfactory. Oil content material and fatty acid composition have also been centered the usage of CRISPR/Cas9 genome enhancing. Knockdown of BnSFAR4 and BnSFAR5 genes in rapeseed has increased seed oil content material without unfavorable results on agronomic parameters**.**

Moreover, knockout of the tt8 gene in *Brassica napus* has led to elevated oil and protein content, in conjunction with changes in fatty acid composition, without big yield-associated deficiency. CRISPR/Cas genome enhancing has additionally been instrumental in investigating crop domestication and specific crop features. For example, deletion of the MYB transcription element gene the use of CRISPR/Cas9 rendered cotton fiberless, losing light on the genetic mechanisms underlying cotton fiber development. In precise CRISPR/Cas genome enhancing offers extraordinary opportunities to enhance crop quality by means of concentrated on particular genes worried in secondary metabolism, carbohydrate and protein composition, oil content, and specific crop capabilities, ultimately contributing to improved agricultural productivity and human health [30].

10. CONCLUSION

Despite its exceedingly current discovery, the CRISPR/Cas technique has unexpectedly risen to prominence as one of the most effective tools in molecular biology research. Indeed, CRISPR/Cas era is poised to revolutionize crop enhancement efforts, emerging as a key breeding technology on this regard. Over the beyond eight years, CRISPR/Cas has seen unprecedented growth and utilization in biological and medicinal studies, outpacing the improvement and adoption of every other generation in current reminiscence. However, notwithstanding its exceptional progress, numerous demanding situations and unresolved problems continue to be earlier than CRISPR/Cas can acquire substantial use. Addressing those challenges can be crucial in harnessing the total ability of CRISPR/Cas-primarily based genome enhancing and making sure its broader adoption throughout diverse fields. Nonetheless, the fast improvements and transformative ability of CRISPR/Cas technology underscore its status as a groundbreaking tool with profound implications for the destiny of medical inquiry, agriculture, and medication.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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