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Application of Crispr-Cas 9 in Food and Agriculture Science: A Narrative Review

Paroma Arefin[1]* , Md Shehan Habib[2], Tamjid-Us-Sakib[3], Md Murshed Hasan Sarkar[1], Md Zafar As Sadiq[1], Subarna Sandhani Dey[1], Farhana Boby[1], Md Shimul Parvaz[1], Md Saidul Arefin[4], Md Ibrahim[1]

[1] *Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi, Bangladesh*

[2]*Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram, Bangladesh*

[3]*Institute of Mining, Mineralogy and Metallurgy, Bangladesh Council of Scientific and Industrial Research, Bangladesh*

[4]*Institute of Nutrition and Food Science, University of Dhaka, Bangladesh*

*Corresponding author: Paroma Arefin, Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi, Bangladesh.

Abstract

Genetic diversity is the foundation for introducing plant development programs. Scientists and breeders used various methods, ranging from modern crossing to classical biotechnologies, to address the restricted incidence of natural mutations. Incomplete gene disruption or spontaneous injection of transgenes into plant genomes also occurs in earlier generations of knockout and gain-offunction technologies. Recent advances in genetic engineering has brought numerous benefits to the food and agriculture sector by improving the essential features of agricultural features. The CRISPR / Cas9 technology emerges from a bacterial immune system of type II and represents a new age of selective genome editing technology that can be spread to almost all species. In this paper, we have focused on advancements in the utilization of the CRISPR-Cas9 system in the food and agricultural industry, particularly in the development of resistant crops with improved quality and productivity.

Keywords: *CRISPR / Cas9 technology; Application in food and agriculture; Gene editing; Crop improvement; Future perspectives.*

Introduction

Over one billion people in today's world are suffering from chronic malnutrition, while our agricultural production are declining at the same time, compounded by the loss of biodiversity and the growing challenges of climate change. With the global population expected to reach 9 billion by 2050, contemporary agriculture will face huge challenges, requiring higher yields and improved quality crops, and requiring less inputs [1-3]. While conventional breeding is presently the most commonly used approach to plant improvement, it is labor intensive and typically takes many years to advance from the initial stages of sampling phenotypes and genotypes to the first crosses into industrial varieties.

The food industry has a 2050 time limit for developing and expanding the food supply chain in order to support the growing population in the world. Progress is required for this reason through crops, livestock and microbes [4,5]. Researchers have been trying to take the steps needed to achieve this milestone since 2005, but efforts have failed. The food production sector is now in a position to make some of its most exciting developments since the Green Revolution with the advent of CRISPRs and CRISPR (Cas) proteins [4,6].

CRISPR/Cas9 Vectors for Gene Editing in Plants

Cas9 and sgRNA expression within the targeted cell is sufficient to modify plant genomes. The promoters. AtU6 (Arabidopsis); TaU6 (wheat); OsU6 or OsU3 (rice)] of plant-specific RNA polymerase III are used to express Cas9 and gRNA in plant systems. In plant systems, there are many commercially produced vectors for expressing variants of Cas9 or Cas9 and gRNAs. Addgene is a national, non-profit

plasmid database that can currently make available in binary vectors2 more than 30 empty gRNA backbones. The empty gRNA backbones have plant promoter RNA polymerase III and gRNA scaffolds to which a scientist may be interested in adding gRNA [4, 7-12].

CRISPR for Crop Improvement

To date, CRISPR / Cas9 gene editing system has been implemented in nearly 20 plant species for various features including yield enhancement, control of biotic and abiotic stress. Many of the published papers are regarded proof-of-concept studies because they characterize the implementation of the CRISPR / Cas9 system by knocking out particular reported genes that play a significant role in processes that tolerate abiotic or biotic pressure [12-18]. Biotic stress imposed by pathogenic micro-organisms pose severe challenges in the development of disease-resistant crops and account for more than 42% of potential yield loss and contribute to 15% of global declines in food production. CRISPR/Cas9-based genome editing has been utilized to increase crop disease resistance and also to improve tolerance to major abiotic stresses like drought and salinity [19-25].

Monocots

Rice

Rice (Oryza sativa) is a major staple food plant for over half of the world's population and is well studied and serves as a model for monocots due to its small genome size. In the recent past, numerous studies have shown the application of the CRISPR-based approach to genome editing in rice and few studies have documented the use of genome editing to enhance rice plant biotic and abiotic pressure [9,26-30].

Wheat

Wheat is an essential grain of cereals, cultivated as a staple food crop worldwide. It has also been shown that the CRISPR TaMLO knockout gives resistance to powdery mildew disease caused by Blumeria graminis f. sp. (Btg) Tritici. Of the transgenic lines tested for restriction enzyme digestion using T7 endonuclease I (T7E1) of the 72 T0 knockout MLO wheat homoeolog (TaMLO-A), four lines have been found to be edited for the restriction enzyme site. Effective methods of construction delivery may reduce the number of transgenic lines acquired or increase them [31-40].

T-DNA-based delivery systems are widely used for SSN and gRNA introduction. Nevertheless, amplicons based on DNA-virus tend to result in several improvements in the efficacy of gene targeting. The use of geminiviral wheat-based DNA replicates Wheat Dwarf Virus (WDV)] for transient and clear expression of CRISPR / Cas9 cassettes resulted in a 12-fold increase in endogenous ubiquitin gene expression in hexaploid wheat. A possible approach for genetic engineering of complex genomes in the future will be high frequency gene targeting using WDV-based DNA replicates [41-55]. Multiplexed genome editing based on CRISPR / Cas9 has been shown to edit many essential agronomic features simultaneously for model crops. The mutation frequency and heritability developed in hexaploidy wheat via multiplexed genome editing. In this study, three wheat genes, TaGW2 (a negative grain regulator), TaLpx-1 (lipoxygenase that provides resistance to Fusarium graminearum) and TaMLO (loss of function, confer resistance to powdery mildew resistance) were targeted using three gRNAs combined in a tRNA spaced polycistronic cassette under a single TaU3 promoter transcriptional control [40,42,45,56].

Maize

Maize (Zea mays) is a major cereal crop, with phytic acid accounting for more than 70% of maize seed. It is believed to be anti-nutritional because it is not digested by mono-gastric animals and is also an environmental pollutant. Targeted gene removal is involved in the synthesis of phytic acids (ZmIPK1A, ZmIPK, and ZmMRP4) in Z. Oh, mays. Similarly, gene editing of the phytoene synthase gene (PSY1) was performed using the U6 snRNA maize promoter. PSY1 is involved in carotenoid biosynthesis and its mutant (psy1) results in white seedlings and albino seedlings [31-45]. Of the fifty-two T0 lines obtained through agrobacterium-mediated transformation, seven lines were reported to carry the psy1 knockout trait and all seven lines were deeply sequenced to understand the type of variation and evaluate the efficiency of the mutation. No off-target sites were edited and stable psy1 mutants were achieved.

Knockout of Zmzb7 results in albino plant, with the sgRNA designed to target a region in the eighth exon of Zmzb7 and maize U3 promoter was used for expression. Following Agrobacterium-mediated transformation of maize embryos, T0 lines were found to show a 31% mutation efficiency. Current high yielding maize varieties benefit from the production of hybrid maize seeds, and hybrid maize production requires sterilization to avoid self-fertilization. CRISPR / Cas9 approach targeted maize thermo sensitive male-sterile 5 (ZmTMS5), known to cause male sterility. The gene was knocked out by three gRNAs, with one targeting the first exon and the other two targeting the second exon. Mutation efficiency was investigated using PCR / restriction enzyme assays in maize protoplasts. Mutational efficiency analysis revealed that there were no off targets for the sgRNA targeting the first exon, while the other two sgRNAs

had off targets in the maize genome.Two genome edited variants (ARGOS8-v1 and ARGOS8-v2) were used for the production of hybrids and evaluated in the field in multi-location trials. Improved yield under stress observed for the variant hybrid than the wild-type [56,57].

Genome Editing in Other Monocots

In addition to model crops, the genome editing approach to CRISPR / Cas9 has been extended to other monocotype crops to enhance essential features. A group of five gRNAs were engineered to use both particle bombardment and agrobacterium-mediated transformation to knock out ENGase. Mutant barley lines T0 and T1 were genotyped with 78 percent mutational efficiency. Such plants will be useful for the study of genes in functional genetics. Recently, the modification of CRISPR / Cas9 has been shown in banana cv. Rasthali of the gene of phytoene desaturase (RAS-PDS) involved in the biosynthesis of carotenoids. Thirteen mutant lines formed by Knockout RAS-PDS in bananas using CRISPR were tested for carotenoid and chlorophyll content.

Dicots

Soybean:

Soybean (Glycine max), one of the most essential crops of seed oil with a high protein content. Additionally, the seed contains a variety of physiologically active substances that benefit people. CRISPR / Cas9-mediated soybean genome editing was active using a single transgen (bar) sgRNA and six sgRNAs targeting different sites of two endogenous soybean genes (GmFEI2 and GmSHR) and evaluating the effectiveness of sgRNAs in a hairy root system. In soybean chromosome 4 (DD20 and DD43), selective mutagenesis of two genomic sites resulted in minor deletions and insertions. The role of a dominant nodulation restriction gene in soybean, Rj4, that inhibits nodulation by many strains of Bradyrhizobium elkanii was shown through both complementation and CRISPR/Cas9-mediated gene knockout experiments.

In Phytophthora sojae, CRISPR has been used to kill the pathogenic virulence gene (Avr4/6). The homologous gene substitution of Avr4/6 with a marker gene (NPT II) induced by the CRISPR / Cas9 method highlighted the contribution of the virulence gene to the pathogen identification by plants containing the soybean R gene loci, Rps4 and Rps6. The soybean flowering gene CRISPR knockout, GmFT2, was stably heritable in the subsequent T2 generation, with homozygous GmFT2a mutants exhibiting late flowering under both long-day and short-day conditions [58-65].

Tomato

Tomato (Solanum lycopersicum L.), an economically important crop, is an ideal candidate for testing CRISPR / Cas9 gene editing due to the availability of effective transformation methodologies, functional genomic characterization and substantial quality improvement background. Tomato (Solanum lycopersicum) is both an important food crop and a model plant species that has been widely used to study gene function, especially as it relates to the biology of fruits. This duality of purpose combined with readily available resources (mutant populations, sequences of genomes, methodology of transformation) makes tomato an ideal candidate for gene editing [11-35]. The CRISPR/Cas9 system routinely used in our laboratory has been applied to various tomato genotypes and the wild species, Solanum pimpinellifolium. The vector system is based on Golden Gate cloning techniques. Cassettes that contain the neomycin phosphotransferase II (NPTII) selectable marker gene that confers resistance to kanamycin, a human codon-optimized Cas9 driven by the CaMV 35S promoter, and guide RNA (gRNA) under control of the Arabidopsis U6 polymerase promoter are assembled into a T-DNA vector. Generally, CRISPR/Cas9 constructs that contain two gRNAs per gene target is used. However, inclusion of up to eight gRNAs to simultaneously target multiple genes and regions has been succesful yet. Introduction of CRISPR-/Cas9-designed constructs into tomato is accomplished by transformation methodology based on Agrobacterium tumefaciens infection of young cotyledon sections and selection on kanamycin-containing medium based on the presence of the NPTII gene [63-70].

Potato

Potato is an important food crop for world food security and, with climate change, it is vital that potato breeds be adapted and that breeding materials be established that can be used to expand the area in which they are grown. Potato starch quality is an important area of research in many of its food applications. The waxy genotype was developed in hexaploid potato by mutating granule-bound starch synthase (GBSS) gene using CMGE. Characterization of starch in genome-edited lines revealed only the presence of amylopectin, with a complete lack of amylose, confirming the knock-out of all four alleles of GBSS .Similarly, multi-allelic mutagenesis has been achieved in potato by mutating ACETOLACTATE SYNTHASE1 (StALS1).

Citrus

Xcc-facilitated SpCas9/sgRNA and SaCas9/sgRNA agroinfiltration were documented in sweet orange and Citrus paradisi, both targeting the Phytoene desaturase, CsPDS and CpPD genes. Improving the resistance of citrus cankers was made possible by targeted modification of the LATERAL

ORGAN BOUNDARIES (CsLOB1) gene's 5' regulatory region. CsLOB1 is the susceptibility gene for citrus canker and plays a critical role in fostering pathogen development and the formation of erumptive pustules [31-36]. Different alleles of CsLOB1 contain the effector-binding element (EBEPthA4). In promoter disrupted CsLOB1, which targets the effector binding element, an increased resistance to citrus canker is observed. A high degree of resistance was provided by deleting the entire EBEPthA4 sequence from both CsLOB1 alleles. In Wanjincheng orange, promoting editing of CsLOB1 alone was necessary to enhance the resistance of citrus canker. Mutation of the coding region of the two susceptibility gene alleles CsLOB1 created citrus cancer resistant in Duncan grapefruit. Fast and efficient genome editing of citrus was documented using the PDS-focused Arabidopsis YAO promoter, indicating that Arabidopsis YAO promoter could drive Cas9 expression for efficient gene editing in the early stages of gene editing [4-6,31-45].

Grape

Grape is an economically valuable fruit, with breeders targeting numerous quality fruit characteristics such as aroma, disease and resistance to abiotic stress, fruit size and skin color. For' Chardonnay ' suspension cells and regenerated grape plantlets, selective genome editing of the L-idonate dehydrogenase gene (IdnDH) is used. In the tested putative off-target sites, no off-target mutations were detected, suggesting high specificity of the CRISPR / Cas9 system in grape genome editing. Most of the mutations found in the transgenic cell mass were either 1-bp insertions or 1-to3-nucleotide deletions followed. Albino leaves resulted in targeted mutagenesis of grape phytoene desaturase (VvPDS) [45-55].

The proportion of mutated cells was higher for older leaves because of either increased incidence of DSBs or faulty repair mechanisms for older leaves. In the widespread grape plant Vitis vinifera, there are five types of CRISPR / Cas9 target sites for potential genome editing. It has been shown that editing the powdery mildew susceptibility gene MLO-7 with purified CRISPR / Cas9 ribonucleoproteins (RNPs) as delivery particles in grape protoplasts is successful. The targeted mutagenesis of VvWRKY52, a gene of transcription factor, has explained its function in biotic stress responses [55,56].

Genome Editing in Other Dicots

CRISPR / Cas9 is a transformative method for selective genetic changes. In plants, after gene editing, high mutation efficiencies have been recorded in primary transformants. Many of the mutations studied, however, are somatic and thus not heritable. Knockout of the gene 9-cis-EPOXYCAROTENOID DIOXYGENASE4 (NCED4) in lettuce (Lactuca sativa) cvs (coding for the first step of abscisic acid biosynthesis). Salinas and Cobham Green, with seeds of both cultivars capable of > 70% germination efficiencies at 37 °C, improves seed germination at high temperatures. NCED4 knockouts provide a plant-wide selectable phenotype with limited pleiotropic effects. Consequently, targeting NCED4 in a co-editing technique could be used to enrich germline-edited events simply by germinating seeds at high temperatures. Germination thermo tolerance due to NCED4 inactivation provides a useful plant-wide selectable phenotype of pleiotropic growth effects [60-68].

The non-pathogenesis-related PR3 gene from cocoa (TcNPR3) is a protective response suppressor and editing it in cocoa leaves provides increased resistance to infection with the cacao pathogen Phytophthora tropical is and increased downstream gene expression. Reliable carrot genome editing of the flavanone-3-hydroxylase (F3H) anthocyanin biosynthetic pathway gene in a purple-colored callus model was used as a visual marker to recognize effectively edited transformation activities. Targeted mutagenesis of the squamosa promoter binding protein-like 9 (SPL9) gene in Medicago sativa (alfalfa), a model vegetable crop was demonstrated and analyzed in a high-throughput way using droplet digital PCR (ddPCR) and lines showing high mutation rates through enzyme restraint digestion / PCR amplification and sequencing. Compared to other less complex plant genomes, overall editing efficiency in the polyploid alfalfa genome was lower [61-70].

The CRISPR / Cas9 genome editing method used to establish virus resistance in cucumber (Cucumis sativus). Targeted mutation of the recessive gene eukaryotic translation initiation factor 4E(eIF4E) was found to confer immunity to Cucumber vein yellowing virus (CVYV), Zucchini yellow mosaic virus (ZYMV), and Papaya ringspot mosaic virus type-W (PRSV-W). Transgenic watermelon plants with ClPDS mutation sand showed clear or albino mosaic phenotype, suggesting that CMGE is theoretically 100% effective in the production of transgenic watermelon lines. albino kiwifruit plantlets using two editing strategies that targeted the phytoene desaturase gene: the polycistronic tRNA-sgRNA cassette (PTG) (PTG/Cas9) and the traditional CRISPR (CRISPR/Cas9) expression cassette was designed.

CRISPR-Modified Foods Coming in the Future

Currently being researched, the crops mentioned below are at different stages of study, growth and production. It should be remembered that the USDA will consider

everything on this list appropriate, but none has yet been accepted for use by the FDA. Apples are being modified to avoid browning when cut and cabbages are done so to improve growing type. Besides, cornns, coffee, soyabeans , wine, banana, cotton, canola, papaya, casava , alfaalfa, watermelon, sugar-beets etc are undergoing modifications in Crisper-Cas gene. There are more out there, and new CRISPR crops are being studied and cultivated every day.

Future perspectives for the improvement of agriculture

While genome editing has many advantages over traditional plant breeding, its application to horticultural crops still poses some challenges. Molecular and genetic studies in horticultural crops are challenging, hampering the identification of genes that are responsible for desirable traits. It will be essential to sequence the genomes of interesting horticultural crops in order to identify genes associated with desirable traits. The target sequence could be cloned for crops without a reference genome using degenerate primers optimized for retained protein patterns with putative functions related to desirable traits. A good example is the MLO, which has been described in detail in barley; the MLO's phylogenetically conservative nature has facilitated the generation of powdery mildew-resistant plants in wheat, tomato, and strawberry [21-35,46-50].

Once a gene has been established to be edited, scientists need to understand the methods used to deliver editing reagents and the technique to restore the edited mutants. To date, more than 25 plant species of horticulture have been successfully edited, usually with editing reagents delivered via agrobacteria or virus systems, and the edited plants are regenerated via tissue culture in vitro. While transformation and regeneration based on tissue culture is most commonly used for genome editing, most horticultural crops do not have a well-established protocol for transformation and regeneration from tissue culture. In plant transformation, an alternate solution to Agrobacterium transformation based on in vitro tissue culture, it pertains to in vivo explant infection in which the targeted tissues are apical or auxiliary meristems, stigmas, pollens, or inflorescences. This technique has been used effectively to transform the species of tomato and brassica and should be further explored for use in horticultural crops that are recalcitrant to traditional genetic conversion. Additionally, successful genetic transformation of horticultural crops requires the consideration of editing efficiency, which is affected by many factors, such as sgRNA number and GC content, the expression levels of sgRNA and Cas9, and the secondary structure of the paired sgRNA and target sequence. In the future, the editing system should be further optimized in different crop species [48, 53-56].

To obtain transgene-free edited plants, the elimination of foreign DNA fragments (transferred T-DNAs) remains difficult in some highly heterozygous and clonally propagated horticultural species such as potatoes, sweet potatoes and bananas. One choice is to produce several transformants, followed by transgene-free mutant high-throughput screening. This method was used to produce approximately 10% of mutants without foreign DNA. Another approach for transgene-free genome editing is to deliver editing reagents as in vitro transcripts or ribonucleoproteins [56-71].

Conclusion

The scientists are able to precisely and rapidly insert the desired characteristics of new breeding techniques than traditional breeding. Genome editing based on CRISPR / Cas9 is a key advance. A prominent areas of work for the future is the application of genome editing tools for enhancing agricultural output, nutritional value, disease resistance and other traits. Some plant systems have been widely used over the past five years to conduct functional studies, combat biotic and abiotic stresses, and improve other important agricultural features. While several enhancements to this technology will result in higher target quality, most of the work performed is preliminary. However CRISPR / Cas9-based genome editing will gain popularity and become a key technique for proper plant editing, helping to achieve the zero hunger goal and maintaining food for the growing human population.

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*Corresponding author: : Paroma Arefin, Email: paroma.arefin@gmail.com