DOI: https://doi.org/10.54393/df.v6i1.120

DIET FACTOR
Journal of Nutritional & Food Sciences
https://www.dietfactor.com.pk/index.php/df
ISSN (E): 2789-8105, (P): 2789-8091
Volume 6, Issue 1 (Jan-Mar 2025)

Review Article

OPEN ACCESS

Tech-Driven Evolution of Trait Performance in Oilseed Crops: A Contemporary Perspective

Zohaib Younas[1], Ilyas Ahmad[1], Tayyaba Yousaf[1], Syed Aoun Abbas Kazmi[2], Ahmad Hassan[1], Muhammad Younas[1], Muhammad Imran[1], Ubaidur Rahman[1], Maaz Ahmad[1] and Zia Ur Rehman Mashwani[1*]

[1]Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
[2]University Institute of Biochemistry and Biotechnology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

ARTICLE INFO

Keywords:
Clustered Regularly Interspace Short Palindromic Repeats (CRISPR), Oilseed Crops, Gene Editing, Traditional Breeding, Vector Construction, Agrobacterium Mediated Plant Transformation

How to Cite:
Younas, Z., Ahmad, I., Yousaf, T., Kazmi, S. A. A., Hassan, A., Younas, M., Imran, M., Rahman, U., Ahmad, M., & Mashwani, Z. U. R. (2025). Tech-Driven Evolution of Trait Performance in Oilseed Crops: A Contemporary Perspective: Advancements in Gene Editing Techniques. DIET FACTOR (Journal of Nutritional and Food Sciences), 6(1), 02–11. https://doi.org/10.54393/df.v6i1.120

***Corresponding Author:**
Zia Ur Rehman Mashwani
Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
mashwani@uaar.edu.pk

Received date: 27th November, 2024
Revised date: 9th March, 2025
Acceptance date: 12th March, 2025
Published date: 31st March, 2025

ABSTRACT

Targeted nucleases are resilient genomic tools that accurately modify the intended genome of living cells, regulating functioning genes with great precision. Gene editing techniques (GETs), especially CRISPR-cas9, are utilized for genetic manipulation with greater efficacy, versatility, cost-efficiency, and capacity for high-throughput applications in the fields of medicine, biology, agriculture, and biotechnology. It has been successfully used for the treatment of genetic diseases in humans and oilseed crop improvements such as disease resistance, reducing seed shattering, herbicide resistance, and improving oil quality and quantity. The purpose of this review is to summarize the potential application of GETs to bring improvements to oilseed crops. In the current study, three different methodologies to incorporate desire traits in oilseed crops are discussed, mainly for the needs of farmers and consumer demands. The methodologies included conventional plant breeding (CPB), mutagenesis plant breeding (MPB), and the advanced gene editing tool CRISPR-cas9. Ongoing inventions in the agriculture field and in the last decade (ten years) are focused. Results: Mechanistic representation in detail was given for editing plant genomes using various strategies such as PEG-mediated, biolistic, and agro-bacterium-mediated plant transformation. The modification of agricultural crops was required to increase the nation's economic condition. In the future, to overcome food security issues, researchers from multidisciplinary fields can plan their work in oilseed crops or relevant disciplines for the betterment of humanity.

INTRODUCTION

A precise, robust and efficient genome editing technique (GET), CRISPR-cas-9 has revolutionized molecular biology and genetics [1]. CRISPR functions as a part of adaptive immune system in prokaryotes against invading viruses/phages or plasmid [2]. During encounters, small fragments of invaders DNA (spacer sequences) become part of bacterial own's genome known as a CRISPR locus. The bacteria then transcribe the Crispr locus along with newly spacer sequences into mRNA (tracrRNA). Multiple spacers and repeats are interspersed within the CRISPR array to produce a single precursor RNA molecule known as pre-crRNA. RNA polymerase is typically responsible for this transcription. Pre-crRNA is processed by RNAase III by binding at spacer sequences, which cleaves it into gRNA. The sgRNA is generally composed of a 20-bp sequence unique to the target DNA, followed by a brief "NGG" or "NAG" sequence known as the "PAM," which is required for Cas9 protein interaction [3]. Cas9 associates with the guide RNA molecule, typically a synthetic single-guide RNA (sgRNA) that merges the roles of both crRNA and tracrRNA, to

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create the Cas9 complex (Figure 1). Most notably, CRISPR-associated proteins like Cas9 and Cas12a nucleases have been responsible for the fast expansion of genome editing [4]. Addressing more obscure CRISPR-Cas systems in bacteria and archaea is critical because they have the potential to significantly expand the scope of plant gene editing tools [5]. Implementing genetic modifications such as substitutions, insertions, and deletions which improve agronomic features can speed up crop enhancement and breeding efforts [6]. The phylogenetic tree for CRISPR-Cas was categorized into two major classes (1 & 2) along with six types and into 16 subtypes. Cas9 and Cas12a/Cpf1 are most common well-known nucleases used for plant genome medication belongs to class 2 with type II and IV, respectively [7, 8]. Additionally, the CRISPRa (CRISPR activation) and CRISPRi (CRISPR interference) techniques rely on Cas9 with inactivated cutting capabilities (dCas9) coupled with various effector domains to regulate the transcription of target genes. At specific target sites, the CRISPR/Cas9 system induces double-stranded DNA breaks (DSBs). After the DSB is repaired, genetic changes may ensue due to the repair machinery of the cell. Cell-specific DNA repair mechanisms, including the frequently error-prone non-homologous end joining (NHEJ) and the less commonly occurring homology-directed repair (HDR), play a crucial role in fixing double-stranded breaks (DSBs) during genome editing [9]. NHEJ is typically utilized to modify genetic sequences, while HDR can introduce or modify information at a specific genomic locus using carefully designed repair templates [10]. Brassica napus L. (2n = 38) has evolved from ancient interspecific hybridization techniques with two diploid species, Brassica rapa (2n = 20) and Brassica oleracea (2n = 18) [11]. The existence of multiple gene copies with high sequence homology in canola (Brassica napus) complicates the gene function research. If one wants to create a consistent phenotype, it is critical to knock off all homologous genes [12]. Recently, the versatile CRISPR/Cas system has gained prominence for efficiently introducing mutations at multiple gene sites in multi-copy gene knockout studies [6]. Additionally, it was found in literature that genome editing has been successfully used for over 40 species of crops in 25 countries, having the ultimate objective of improving agronomy, food and nutritional quality, and resistance to abiotic stress [13]. Despite its enormous assurance, still six genome-edited crops traits have gotten commercial clearance .

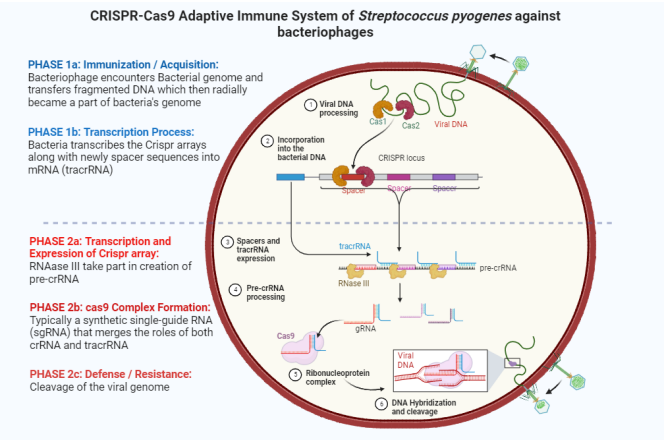

Figure 1: Bacteria Shows Acquired Immunity Against Viral Genome

Application of CRISPR/cas9 in Genome Editing

Over time, the research of gene function has commonly used approaches such as overexpression, silencing, and DNA insertion, all of which necessitate the incorporation of foreign DNA segments into the host genome [14]. Yet, problems occur during plant genetic transformation, especially the danger of unintentional insertions, which lead to regulatory restrictions on transgenic crops as genetically modified organisms. These legislative constraints and potential unforeseen genomic changes impede the progress and utilization of such crops [15]. Researchers are increasingly enthusiastic about genome editing techniques (GETs) owing to their remarkable attributes, including precision, versatility, cost-efficiency, and capacity for high-throughput applications. These GETs have become invaluable for the functional characterization of specific genes in a diverse range of organisms, such as bacteria, plants, animals, and even humans [16-18]. The limitations of GETs extend beyond plant genome modification, as they have proven successful in modifying gene traits in a variety of organisms, including fruit flies, mice, rats, and even in the treatment of human diseases (Figure 2). The error prone changes in hematopoietic stem cells responsible for the cause of hematologic diseases [19, 20]. Successful case studies utilizing CRISPR are reviewed individually [21, 22]. The application of CRISPR/cas9 for genome editing in diverse range of fields as depicted in Figure 2. Contribution of cas9 in biomedical field; A landmark study used CRISPR/Cas9 to repair cataracts in a rat model caused by base deletions. They co-injected mRNA expressing Cas9 and a sgRNA into the fertilized eggs of mice prone to cataracts in this work. The experimental trial was performed on twenty mouse progenies, in which ten mice (45.4%) contained mutant allele. Despite these six mice (27.3%) displayed NHEJ-mediated insertions and deletions (indel) and four (18.1%) underwent HDR-mediated repairs. Surprisingly, all four mice with cataracts fixed by

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HDR induction were entirely healed, as were two of the NHEJ-induced animals. These studies proved CRISPR/Cas9's ability to change the genome for the therapy of hereditary disorders [23, 24]. Another praising contribution to this cas9 tool in field of medicine. Human beings suffer from genetic disease can be cured with CRISPR-cas9 application. It was revealed that (GETs) is a potential contender in treatment of numerous genetic, bacterial and viral diseases. CRISPR-Cas9 offers a very promising method for treating hematological disorders by targeting the HBB gene. Hematopoietic stem and progenitor cells (HSPCs) and induced pluripotent stem cells (iPSCs) derived from patients have shown success with this technique. HBB gene alterations were corrected in these trials, resulting in lower hemoglobin levels and less sickle cell disease. The clinical application of CRISPR-Cas9 in hematological disorders shows its therapeutic promise [19]. Finally, use of CRISPR-cas9 system was addressed for integrating plant genome especially focused on oilseed crops: To address concerns regarding erucic acid in canola oil and its potential health effects; Shi et al., conducted research aimed at reducing erucic acid levels in Canola plants [25]. Using the B. napus cultivar "CY2" as the transgenic recipient, they modified fatty acid compositions by introducing a BnFAE1 fragment driven by napin A promoters and co-culturing hypocotyls with Agrobacterium tumefaciens EHA105. Through seed-specific knockdown of BnFAE1 in CY2, the researchers effectively changed the fatty acid composition, notably reducing erucic acid to less than 3% in the resulting transgenic canola lines. This intervention involved RNAi constructs that successfully interfered with BnFAE1 mRNA levels in F1 hybrid seeds.

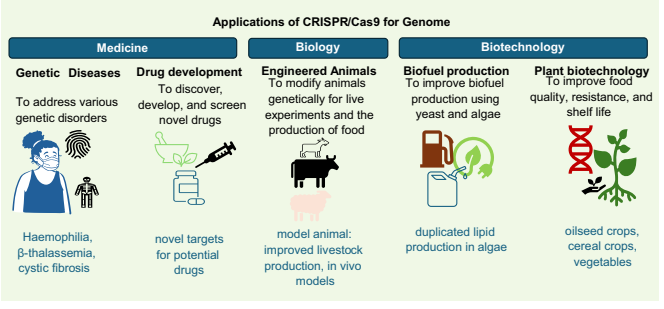

Figure 2: Pictorial Representation of CRISPR/Cas9 Applications in Field of Medicine, Biology and Biotechnology

Biochemistry and De novo Synthesis of Fatty acids (FAs)

It has been described that genes are responsible for controlling oil/seed trait in *Brassica napus*. They developed transgenic depressed lines in which BnFAD2 and BnFAE1 were targeted through using napin A promoter and succeeded. The de-expression of both FAD2 and FAE1 resulting in increment of oleic acid unsaturated fatty acid (OAUFA) and protein profiling in seeds. They also determined that increase in OAUFA is inversely proportional to erucic acid (EA) content. EA content lower in seed of *Brassica napus* when OA content was increased, it improved overall oil nutritional profile. Furthermore, the poly-unsaturation in oleic acid (18:1) to linoleic (C18:2), linolenic (C18:3) enhanced its stability. Meanwhile, the protein content is a major contributor to the meal energy value for feed. The formation of complex molecules such as FAs from precursor units i.e. (sugars and amino acids) referred to as de novo synthesis. The oil is categorized into two main types (1) consumable and (2) non-consumable. In mentioned categories the oil that obtained from *Brassica napus* is not directly consumable before it undergoes through several analysis and process. The seed oil is predominately composed of TAGs, which are essential for human nutrition. To check the detailed study about FAs synthesis, it occurred in two different organelles such as plasmid and cytoplasm. Acetyl-CoA is the precursor unit for UFA synthesis which undergoes through series of reactions resulting in various intermediate compounds. All the reactions are carried by specific enzyme [26]. The FAs synthesis is driven by Acetyl-CoA carboxylase enzyme, in which acetyl-CoA is converted to first intermediate compound malonyl-CoA. The malonyl-CoA is then converted into other unstable intermediate compound known is stearic acid in presence of ACP. The ACP is responsible for the addition of two carbon groups into newly synthesized compound. Furthermore, the synthesis of FA proceeded and resulted in the formation of oleic acid. The FAB2 enzymes drive the reaction which occurred in plastid of plant cell. Despite this, the chain elongation formation in endoplasmic reticulum or cytosol of plant cell depicted in figure 3. The oleic acid is then converted into either eicosenoic acid synthesis in the present of fatty acid elongase (FAE1) or linoleic acid with the help of fatty acid dehydrogenase (FAD2).

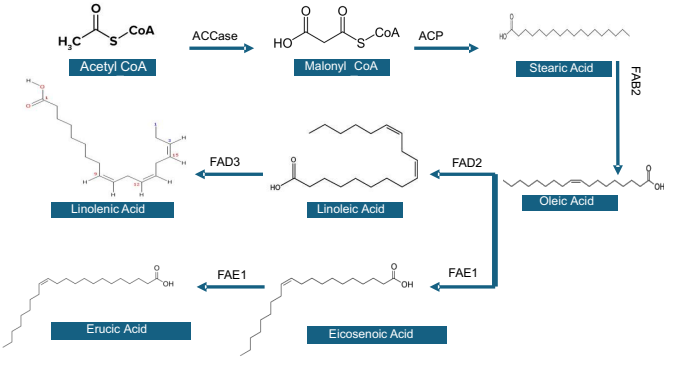

Figure 3: Mechanism for Fatty Acid Synthesis in *Brassica napus* L. The Synthesis Process from Acetyl-CoA to Oleic Acid Occurs in Plastid and Elongation Occurs in Endoplasmic Reticulum

Traditional Breeding Techniques (TBT)

The inherent genetic variety found in crop cultivars,

DOI: https://doi.org/10.54393/df.v6i1.120

landraces, and their wild relatives offers the genetic diversity that's needed for plant breeding and agricultural development. Molecular research elucidated that genes play a critical role in casting plant attributes including shoot branching, tiller count, flowering period, grain production, grain size, nutrient utilization efficiency, and tolerance to both environmental and biological challenges [27, 28]. In the face of environmental concerns, shrinking farmlands, and depleting groundwater resources, plant breeding innovations are crucial to boost crop yields and establish resilient agriculture to meet the consumer demands. In modern-day farming, key crop-enhancing methods such as conventional plant breeding (CPB) or hybridization, mutation breeding (MPB), and transgenic techniques are indispensable [29]. In this review, three different strategies are discussed such as 1) Conventional plant breeding (CPB), 2) Mutagenesis plant breeding (MPB) and 3) Transgenic using CRISPR-cas9 system. Traditionally, CPB has been used in agriculture to develop new plant varieties by selecting and crossing parent plants that exhibit desired traits [30]. Using this technique, plants with certain qualities, such as disease resistance, higher yields, and better yield quality are intentionally pollinated together. It is frequently most laborious and time-consuming method. This repeated selecting and backcrosses cycle tries to produce new plant varieties with an assortment of beneficial traits that meet the demands of consumers, farmers, and changing environmental circumstances [31, 32]. It is concluded that CBP takes around a ten-year period to introduce favorable alleles while optimizing polymorphism effectiveness through genetic recombination. Interestingly, unlike genetic modification procedures, CBP depends on natural genetic diversity and does not entail the incorporation of foreign genes into the plant's DNA. Secondly, mutagenesis plant breeding is a technique used in agriculture to induce genetic variations in plants by exposing them to physical mutagenic agents such as radiation or chemicals. Recently, research induced mutation using various doses of irradiation ranges from (25 to 300 Gy) heavy ion beam (HIB). There is limited research on the mutation features caused by various HIB dosages employing low-generation (M1 - M2) mutants without phenotypic bias [33]. They proceeded with their experiment up to 6th generation and concluded that M3 and M6 have highest number of phenotypic mutants [34, 35]. Based on research, mutagenesis plant breeding takes years to produce mutant plants with desirable traits. It would be noted that during mutagenesis, personal care must be kept to preference and protective kits should be used to protect oneself from HIB. Finally, the CRISPR-cas9 system, a novel tool of gene editing utilization for crop improvement, got fame due to its success rate, less off-targeting effect and high precision rate. Interestingly, gene editing tools might help to accelerate the process [36]. Braatz *et al.*, revealed that CRISPR/Cas9 was initially used to improve shattering and disease resistance in canola by targeting a particular gene (BnALC) [37]. Extensive research (2017 to current day) has proved the maturity of technology, promoting substantial improvements in Brassica napus breeding while offering an understanding of gene function, molecular processes, and prospective routes for seed oil enhancement [38, 39].

CRISPR/cas9 Delivery Platform

Current plant genome editing techniques (GETs), based on established plant transformation methods are presently limited to a few species. It may sound easier to transport CRISPR-cas9 systems into rigid cell structures, but it is quite challenging [40]. Plants face challenges with DNA template delivery, limiting nuclease-initiated homology-directed repair (HDR). Further, the researchers faced difficulties in identifying and successfully retrieving targeted plants. To overcome this, they utilize CBEs and ABEs, enabling specific transitions but not transversions, insertions, or deletions. Moreover, transgene-free altered plants must be produced for commercial reasons, which necessitates reagents and methods of delivery that are DNA-free. Plant cells can be transformed using PEG, Agrobacterium, bombardment, or biolistic methods [41, 42]. The mechanistic approaches for delivery of CRISPER-cas9 system or reagents into plants are given below in detailed (Figure 5).

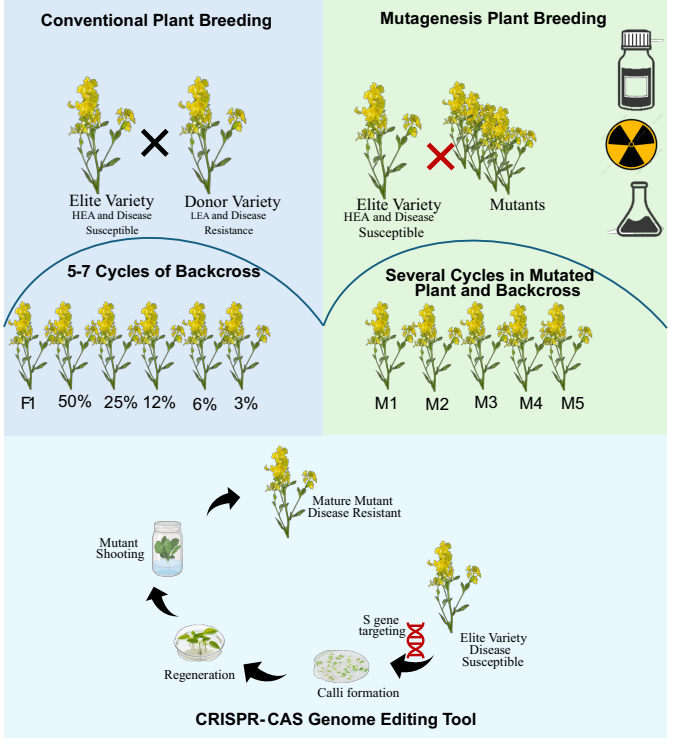

Figure 4: Breeding Techniques for Oilseed Crop Improvement

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PEG Mediated Delivery of CRISPR Reagents

Plant, fungal, bacterial, or archaeon protoplasts are cells that consist of their cell walls which are destroyed by plasmolysis, retaining just protoplasm and plasma membrane [43]. The isolation of protoplasts increases their susceptibility to gene delivery methods. The DNA, RNA, RNPs incorporate into host targeted sites or cell wall free protoplast transports with help of nanocarriers [44]. The main issues with PEG-mediated transformation are that it may cause toxicity or cytotoxicity. To tackle this issue, various transformation methods like electroporation, lipofection, and biolistics have been proposed [45]. Protoplast transformation for genome editing is constrained by explant type, protoplast quality, and the fragility of protoplasts, necessitating careful handling. A key challenge in PEG-mediated transformation is setting up optimal conditions for explants, culture types, and light exposure. It was difficult for researchers to reduce oxidative stress during isolation and culture. The incorporation of CRISPR elements into plant cells, notably oilseed crops, is a hot field of study with the goal of improving agricultural attributes like production, disease resistance, and nutritional value. Designing markers associated with regeneration and proto-clonal variation could be based on factors like chromocenter (re)assembly, ROS activity, DNA methylation, histone methylation, phytohormone ratios, or gene expression. This approach has the potential to create custom and genotype-specific regeneration protocols, ensuring the broad applicability of protoplast-based techniques.

Biolistic Transformation

Biolistics or particle bombardment is a prevalent technique used to transform plants that are resistant to Agrobacterium infection. This technique involves propelling gold or tungsten particles (0.6 µm and 1.0 µm) coated with DNA at high speeds into plant tissue, allowing the DNA to enter plant cells [46]. Reagents are typically applied to microcarriers in an aqueous solution and subsequently precipitated using chemicals such as spermidine/CaCl2/PEG, glycogen, or a cationic lipid reagent. It is extensively used for delivering plasmid DNA, ssDNA, RNA and (ribonucleoprotein) RNPs into chloroplasts and mitochondria. Once inside, the DNA, RNPs separates from the particles, leading to transient expression or stable integration into the host genome. In one work, Luo et al., administered TALEN proteins, ALS2T1L and ALS2T1R, to a region 306 bp downstream of the NbALS2 genes in N. benthamiana protoplasts resulting in a 1.4% mutation frequency. Unlike Agrobacterium, biolistics physical DNA delivery bypasses host-range limitations. The main disadvantages of delivery include uncontrolled integration at many genomic loci when delivered as DNA, laboriousness, and embryo rejuvenation. A significant issue with this tool was found to be the inability to control bombardment sites when targeting organelles like the cytoplasm, nucleus, mitochondria, and plastids [46]. A dual-barreled gene cannon has been created alongside cell counting software to standardize bombardment tests. By adding an internal standard, therefore reducing the usual deviation between bombardments. These improvements attempt to increase the consistency and accuracy of gene delivery systems [47]. It has effectively been delivered to various crop plants such as canola, corn, cotton, soybean, and wheat. This method offers a versatile approach for successful genome editing across multiple plant species [48]. Standardized growth mediums, tissue culturing, vector designing, Selectable markers, gene of interest, Agrobacterium medium plant transformation, mutants' generation all steps carefully needed for a successful outcome. It is summarized that, no doubt these gene editing tools may save time but also lead to productive outcomes also.

Table 1: Biolistic Method for CRISPR/Cas9 System Delivery in Oilseed Crop

Oilseed Crops	CRISPR-cas9 Vector	Selectable Markers	Targeted Genes	Targeted Outcomes	References
Glycine max	QC799 and RTW831	Pat	ALS1	Indels, Rep (HDR) & Editing	Ran *et al.*, 2017
Glycine max	QC810 and RTW830, QC799 and RTW831	*HptII*	DD20, DD43	Indels, Rep (HDR)	Li *et al.*, 2015
Brassica napus	pP1C-4	-	GhCLA1, GhVP	Indels, Rep (HDR)	Chen *et al.*, 2017
Gossypium hirsutum	pCAMBIA-1300	hygromycin	FAE1, FAD2	Indels, Editing	Shi *et al.*, 2022

Agrobacterium-Mediated Plant Transformation

Genetic transformation techniques have advanced crop improvement by integrating new genes, fulfilling the demand for high-yield, quality crops with features such as enhanced oil production, herbicide tolerance, and disease resistance. Agrobacterium transformation remains key for delivering gene-editing tools like CRISPR/Cas variants, base editing, and prime editing into plants. This transformation technique may process through various steps. Initially, research initiated with identification of the specific genes within the plant genome that are to be modified using CRISPR/Cas9. This step proceeded after as development of the CRISPR/Cas9 constructs that carry the guide RNA (gRNA) targeting the chosen gene(s) and the Cas9 nuclease. Furthermore, incorporation of the designed CRISPR/Cas9 constructs into Agrobacterium tumefaciens, a common method to transfer genetic

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material to plant cells is mandatory. The tricky step is following by incubation of the Agrobacterium tumefaciens containing the CRISPR/Cas9 constructs with plant cells to facilitate the transfer of the genetic material. Selection of plant cells that have incorporated the CRISPR/Cas9 constructs and initiating the regeneration process to grow these transformed cells into whole plants [49].

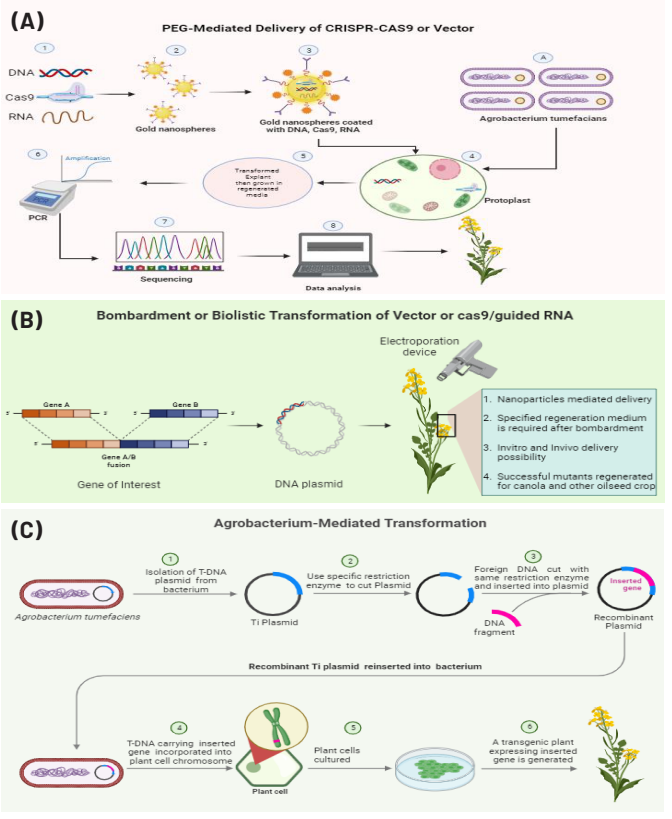

Figure 5: The mechanism for CRISPR/cas9 system delivery platforms using PEG-mediated, Biolistic and Agrobacterium mediated transformation. A) In PEG mediated CRISPR-cas9 delivery onto gold coated nanospheres was carried with gene gun and transferred into protoplast via PEG. In process proceeded to delivered this in newly explant formation and then placed these explants in newly prepared regeneration medium for shooting. Then the PCR was done to multiply the desired sequences and sequencing will be performed. The data analysis regarding this will be visualized on screen for confirmation. B) In biolistic mechanism, the gene of interest along with cas9 is carried out in vitro and then incorporate into protoplast. The foliar targeting with nanoparticles mediated delivery of cas9 should be carried out using gene gun directly. C) The step wise Agrobacterium mediated plant transformation is described well in schematic representation.

Modification in Oilseed Crop by Base Editing and Prime Editing Utilization

"Base editing" offers a precise method for changing nucleotides without disrupting genes or needing donor templates. Base editors offer a platform to change one base to another facilitated by the cytosine or adenosine deaminase domain. The cytidine deaminase enzyme transforms cytosine into uracil by removing an amino group, creating a U-G mismatch. DNA repair pathways then resolve this mismatch by forming U-A base pairs (Figure 6). Following this, a T is added to the new strand, leading to T-A base pairs, causing programmed C-G to T-A conversion. In 2016, Harvard University researchers, led by David Liu, developed BE1—a base editor combining a rat APOBEC1 cytidine deaminase enzyme with a dCas9 using a 16 amino acid XTEN linker [50]. This method allows for single-base modifications, potentially leading to beneficial trait variations in agricultural plants, thereby aiding in crop development. By rectifying single-base modifications or single nucleotide polymorphisms (SNPs) without disrupting the gene, the base-editing technique minimizes indels [51]. It has been revealed that Canola has employed base editing strategies like CBE and ABE. Herbicide-resistant canola has been produced using CRISPR/Cas9-mediated CBE, which successfully introduces C to T conversion in canola sgRNA targets, simplifying weed control for canola production [52]. This efficient method shows promise in establishing new characteristics in crucial agricultural crops, greatly contributing to food security. The primary editor has found extensive use in cereal and vegetable crop breeding research, yet its application in oilseed crops has been limited [53]. This limit is set because our study primarily aimed to gather optimal information on utilizing the CRISPR-Cas9 system for oilseed crop advancement.

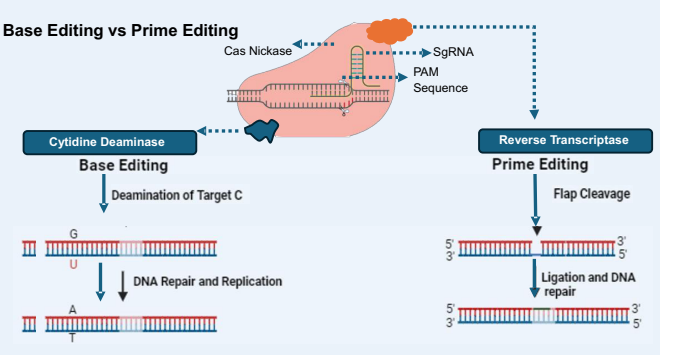

Figure 6: Pictorial Representation of Genome Editing through Base Editing and Prime Editing Utilization for Oilseed Crop Modification

Ethical Concerns in GETS (CRISPR/cas9)

The ability of gene editing technologies like CRISPR-Cas9 to modify the genetic makeup of living organisms, including humans, animals, and plants, has raised several ethical issues. There are many ethically concerns related to this CRISPR/cas9 system described as; Firstly, Off Target Effects: These off-target effects might have unanticipated

DOI: https://doi.org/10.54393/df.v6i1.120

health ramifications in people receiving CRISPR-based medicines. The major concerns related to humans might be later it poses a risk of genetic mosaicism [54-56]. Secondly, Germline Editing: It is extremely ethically problematic to edit the germline, the genetic material that is passed on to future generations. It raises concerns about "designer babies" and genetic enhancement if one alters a person's germline. The third is Environmental Impact: Changing in the genetic makeup of plants results in bioethical issues such as disruption of ecological balance [57]. Fourth is related to Access and Equity: A study proposed issues regarding fair accessibility to genome editing technology. Will genetic improvements be available primarily to the vibrant, resulting in inequities and societal divisions? Moreover, number five is linked to Dual Use Concerns: There is a risk that the same technology used for beneficial purposes could be misused for nefarious purposes, such as creating bioengineered bioweapons. Finally, Genetic Diversity: Genome editing can reduce genetic diversity if used on a large scale, potentially making populations more vulnerable to certain diseases and reducing adaptability [58].

Future Perspective of CRISPR/cas9 Techniques

Plant genome editing will benefit from the use of CRISPR/Cas due to its multiplexing, high throughput editing, and ability to rearrange chromosomes and modify epigenomes. Despite their ease of introduction into plant organelles, Cas nucleases and gRNAs cannot yet be used to edit plastomes and chondriomes. The ongoing study on oilseed crops strives to offer a fresh opportunity for upcoming researchers to contribute to this field. Till now, there are no soyabean varieties which show resistance to abiotic stresses. Likewise, a research gap exists in the realm of hybrid canola varieties, which confront challenges concerning elevated erucic acid levels and undesirable glucosinolate composition with anti-nutritional properties. Moreover, Cotton is a water-intensive crop, and its cultivation often exacerbates water scarcity, especially in regions where water resources are limited. Developing a drought-tolerant cotton variety has the potential to address this problem. Achieving this could involve inducing mutations in the genome to integrate the desired gene of interest via agrobacterium-mediated plant transformation. Further, the CRISPR/Cas9 system has great potential to target cancer causing viruses and genetic disorders. "Last but not least" CRISPR system has significantly influenced cancer research and is poised to maintain an indispensable role in the times ahead.

CONCLUSIONS

New technologies often replace traditional ones due to their higher success rates in clinical trials or ongoing experiments aimed at addressing these concerns. Traditional plant breeding requires several years to achieve desired goals related to food security and environmental concerns, such as developing traits like herbicide resistance, drought resistance, salt soil tolerance, and resistance against infectious diseases. Moreover, when feasible, the commercialization of products or food supplies includes the adoption of advanced techniques within their specific domains. Recently, all of this has become achievable through the implementation of the CRISPR/Cas9 system. In fundamental research, CRISPR/Cas has proven invaluable, especially in gene targeting, knockouts, and gene expression control.

Authors Contribution

Conceptualization: ZURM
Methodology: ZY, TY, SAAK, MY, MI
Formal analysis: IA
Writing review and editing: ZY, IA, TY, AHM MY, MI, UR, MA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Sampson TR, Saroj SD, Llewellyn AC, Tzeng YL, Weiss DS. A CRISPR/Cas System Mediates Bacterial Innate Immune Evasion and Virulence. Nature. 2013; 497(7448): 254-257. doi: 10.1038/nature12048.

[2] Sorek R, Lawrence CM, Wiedenheft B. CRISPR-Mediated Adaptive Immune Systems in Bacteria and Archaea. Annual Review of Biochemistry. 2013; 82: 237-266. doi: 10.1146/annurev-biochem-072911-172315.

[3] Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA. DNA Interrogation by the CRISPR RNA-Guided Endonuclease Cas9. Biophysical Journal. 2014; 106(2): 695a. doi: 10.1016/j.bpj.2013.11.3848.

[4] Burstein D, Harrington LB, Strutt SC, Probst AJ, Anantharaman K, Thomas BC, *et al.* New CRISPR-Cas Systems from Uncultivated Microbes. Nature. 2017; 542(7640): 237-241. doi: 10.1038/nature21059.

[5] Puchta H, Jiang J, Wang K, Zhao Y. Updates on Gene Editing and its Applications. Plant Physiology. 2022; 188(4): 1725-1730. doi: 10.1093/plphys/kiac032.

[6] Wang ZP, Xing HL, Dong L, Zhang HY, Han CY, Wang XC, *et al.* Egg Cell-Specific Promoter-Controlled CRISPR/Cas9 Efficiently Generates Homozygous Mutants for Multiple Target Genes in Arabidopsis in A

DOI: https://doi.org/10.54393/df.v6i1.120

Single Generation. Genome Biology. 2015; 16: 1-12. doi: 10.1186/s13059-015-0715-0.

[7] Shah SA, Erdmann S, Mojica FJ, Garrett RA. Protospacer Recognition Motifs: Mixed Identities and Functional Diversity. RNA Biology. 2013; 10(5): 891-899. doi: 10.4161/rna.23764.

[8] Leenay RT, Maksimchuk KR, Slotkowski RA, Agrawal RN, Gomaa AA, Briner AE, *et al.* Identifying and Visualizing Functional PAM Diversity Across CRISPR-Cas Systems. Molecular Cell. 2016; 62(1): 137-147. doi: 10.1016/j.molcel.2016.02.031.

[9] Li S and Xia L. Precise Gene Replacement in Plants Through CRISPR/Cas Genome Editing Technology: Status and Future Perspectives. Abiotechnology. 2020; 1: 58-73. doi: 10.1007/s42994-019-00009-7.

[10] Vu TV, Sivankalyani V, Kim EJ, Doan DTH, Tran MT, Kim J, *et al.* Highly Efficient Homology-Directed Repair Using CRISPR/Cpf1-Geminiviral Replicon in Tomato. Plant Biotechnology Journal. 2020; 18(10): 2133-2143. doi: 10.1111/pbi.13373.

[11] Chalhoub B, Denoeud F, Liu S, Parkin IA, Tang H, Wang X, *et al.* Early Allopolyploid Evolution in the Post-Neolithic Brassica napus Oilseed Genome. Science. 2014; 345(6199): 950-953. doi: 10.1126/science.1253435.

[12] Wells R, Trick M, Soumpourou E, Clissold L, Morgan C, Werner P, *et al.* The Control of Seed Oil Polyunsaturate Content in The Polyploid Crop Species Brassica Napus. Molecular Breeding. 2014; 33: 349-362. doi: 10.1007/s11032-013-9954-5.

[13] Menz J, Modrzejewski D, Hartung F, Wilhelm R, Sprink T. Genome Edited Crops Touch the Market: A View on The Global Development and Regulatory Environment. Frontiers in Plant Science. 2020; 11: 586027. doi: 10.3389/fpls.2020.586027.

[14] Bao A, Zhang C, Huang Y, Chen H, Zhou X, Cao D. Genome Editing Technology and Application in Soybean Improvement. Oil Crop Science. 2020; 5(1): 31-40. doi: 10.1016/j.ocsci.2020.03.001.

[15] He J, Zhang K, Tang M, Zhou W, Chen L, Chen Z, *et al.* CRISPR-Based Genome Editing Technology and Its Applications in Oil Crops. Oil Crop Science. 2021; 6(3): 105-113. doi: 10.1016/j.ocsci.2021.07.002.

[16] Gaj T, Gersbach CA, Barbas CF. ZFN, TALEN, and CRISPR/Cas-Based Methods for Genome Engineering. Trends in Biotechnology. 2013; 31(7): 397-405. doi: 10.1016/j.tibtech.2013.04.004.

[17] Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. CRISPR-Assisted Editing of Bacterial Genomes. Nature Biotechnology. 2013; 31(3): 233. doi: 10.1038/nbt.2508.

[18] Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, *et al.* RNA-Guided Human Genome Engineering Via Cas9. Science. 2013; 339(6121): 823-826. doi: 10.1126/science.1232033.

[19] Park SH, Lee CM, Dever DP, Davis TH, Camarena J, Srifa W, *et al.* Highly Efficient Editing of the Beta-Globin Gene in Patient-Derived Hematopoietic Stem and Progenitor Cells to Treat Sickle Cell Disease. Nucleic Acids Research. 2019; 47: 7955-7972. doi: 10.1093/nar/gkz475.

[20] Reimer J, Knöß S, Labuhn M, Charpentier EM, Göhring G, Schlegelberger B, *et al.* CRISPR-Cas9-Induced t(11; 19)/MLL-ENL Translocations Initiate Leukemia in Human Hematopoietic Progenitor Cells In Vivo. Haematologica. 2017; 102(9): 1558. doi: 10.3324/haematol.2017.164046.

[21] Chen K, Wang Y, Zhang R, Zhang H, Gao C. CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. Annual Review of Plant Biology. 2019; 70: 667-697. doi: 10.1146/annurev-arplant-050718-100049.

[22] Bassett AR and Liu JL. CRISPR/Cas9 and Genome Editing in Drosophila. Journal of Genetics and Genomics. 2014; 41(1): 7-19. doi: 10.1016/j.jgg.2013.12.004.

[23] Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome Engineering Using the CRISPR-Cas9 System. Nature Protocols. 2013; 8(11): 2281-2308. doi: 10.1038/nprot.2013.143.

[24] Fu YW, Dai XY, Wang WT, Yang ZX, Zhao JJ, Zhang JP, *et al.* Dynamics and Competition of CRISPR-Cas9 Ribonucleoproteins and AAV Donor-Mediated NHEJ, MMEJ and HDR Editing. Nucleic Acids Research. 2021; 49(2): 969-985. doi: 10.1093/nar/gkaa1251.

[25] Shi J, Lang C, Wu X, Liu R, Zheng T, Zhang D, *et al.* RNAi Knockdown of Fatty Acid Elongase1 Alters Fatty Acid Composition in Brassica napus. Biochemical and Biophysical Research Communications. 2015; 466(3): 518-522. doi: 10.1016/j.bbrc.2015.09.062.

[26] Villanueva-Mejia D and Alvarez JC. Genetic Improvement of Oilseed Crops Using Modern Biotechnology. Advances in Seed Biology. 2017; 295-317. doi: 10.5772/intechopen.70743.

[27] Ashkani S, Rafii MY, Shabanimofrad M, Miah G, Sahebi M, Azizi P,*et al.* Molecular Breeding Strategy and Challenges Towards Improvement of Blast Disease Resistance in Rice Crop. Frontiers in Plant Science. 2015; 6: 886. doi: 10.3389/fpls.2015.00886.

[28] Xu Q, Zhao M, Wu K, Fu X, Liu Q. Emerging Insights into Heterotrimeric G Protein Signaling in Plants. Journal of Genetics and Genomics. 2016; 43(8): 495-502. doi: 10.1016/j.jgg.2016.06.004.

[29] Scheben A, Wolter F, Batley J, Puchta H, Edwards D. Towards CRISPR/Cas Crops-Bringing Together Genomics and Genome Editing. New Phytologist.

DOI: https://doi.org/10.54393/df.v6i1.120

2017; 216(3): 682-698. doi: 10.1111/nph.14702.
[30] Acquaah G. Principles of Plant Genetics and Breeding. John Wiley & Sons. 2009.
[31] Das D, Singha DL, Paswan RR, Chowdhury N, Sharma M, Reddy PS, *et al.* Recent Advancements in CRISPR/Cas Technology for Accelerated Crop Improvement. Planta. 2022; 255(5):109. doi: 10.1007/s00425-022-03894-3.
[32] Jeon D, Kang Y, Lee S, Choi S, Sung Y, Lee TH, *et al.* Digitalizing Breeding in Plants: A New Trend of Next-Generation Breeding Based on Genomic Prediction. Frontiers in Plant Science. 2023; 14: 1092584. doi: 10.3389/fpls.2023.1092584.
[33] Ichida H, Morita R, Shirakawa Y, Hayashi Y, Abe T. Targeted Exome Sequencing of Unselected Heavy-Ion Beam-Irradiated Populations Reveals Less-Biased Mutation Characteristics in the Rice Genome. The Plant Journal. 2019; 98(2): 301-314. doi: 10.1111/tpj.14213.
[34] Yang G, Luo W, Zhang J, Yan X, Du Y, Zhou L, *et al.* Genome-Wide Comparisons of Mutations Induced by Carbon-Ion Beam and Gamma-Rays Irradiation in Rice via Resequencing Multiple Mutants. Frontiers in Plant Science. 2019; 10: 1514. doi: 10.3389/fpls.2019.01514.
[35] Zheng Y, Li S, Huang J, Fu H, Zhou L, Furusawa Y, *et al.* Mutagenic Effect of Three Ion Beams on Rice and Identification of Heritable Mutations by Whole Genome Sequencing. Plants. 2020; 9(5): 551. doi: 10.3390/plants9050551.
[36] Ali E and Zhang K. CRISPR-Mediated Technology for Seed Oil Improvement in Rapeseed: Challenges and Future Perspectives. Frontiers in Plant Science. 2023; 14: 1086847. doi: 10.3389/fpls.2023.1086847.
[37] Braatz J, Harloff HJ, Mascher M, Stein N, Himmelbach A, Jung C. CRISPR-Cas9 Targeted Mutagenesis Leads to Simultaneous Modification of Different Homoeologous Gene Copies in Polyploid Oilseed Rape (Brassica Napus). Plant Physiology. 2017; 174(2): 935-942. doi: 10.1104/pp.17.00426.
[38] Tan Z, Xie Z, Dai L, Zhang Y, Zhao H, Tang S, *et al.* Genome-and Transcriptome-Wide Association Studies Reveal the Genetic Basis and the Breeding History of Seed Glucosinolate Content in Brassica Napus. Plant Biotechnology Journal. 2022; 20(1): 211-225. doi: 10.1111/pbi.13707.
[39] Yan G, Yu P, Tian X, Guo L, Tu J, Shen J, *et al.* DELLA Proteins BnaA6. RGA and BnaC7. RGA Negatively Regulate Fatty Acid Biosynthesis by Interacting with BnaLEC1s in Brassica Napus. Plant Biotechnology Journal. 2021; 19(10): 2011-2026. doi: 10.1111/pbi.13628.
[40] Altpeter F, Springer NM, Bartley LE, Blechl AE, Brutnell TP, Citovsky V, *et al.* Advancing Crop Transformation in the Era of Genome Editing. The Plant Cell. 2016; 28(7): 1510-1520. doi: 10.1105/tpc.16.00196.
[41] Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable Editing of a Target Base in Genomic DNA Without Double-Stranded DNA Cleavage. Nature. 2016; 533(7603): 420-424. doi: 10.1038/nature17946.
[42] Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI. Programmable Base Editing of AT to GC in Genomic DNA Without DNA Cleavage. Nature. 2017; 551(7681): 464-471. doi: 10.1038/nature24644.
[43] Wu S, Zhu H, Liu J, Yang Q, Shao X, Bi F, *et al.* Establishment of a PEG-Mediated Protoplast Transformation System Based on DNA and CRISPR/Cas9 Ribonucleoprotein Complexes for Banana. BMC Plant Biology. 2020; 20: 1-10. doi: 10.1186/s12870-020-02609-8.
[44] Li Z, Liu ZB, Xing A, Moon BP, Koellhoffer JP, Huang L, *et al.* Cas9-Guide RNA Directed Genome Editing in Soybean. Plant Physiology. 2015; 169(2): 960-970. doi: 10.1104/pp.15.00783.
[45] Liang Z, Chen K, Gao C. Biolistic Delivery of CRISPR/Cas9 with Ribonucleoprotein Complex in Wheat. Plant Genome Editing with CRISPR Systems: Methods and Protocols. 2019; 327-335. doi: 10.1007/978-1-4939-8991-1_24.
[46] Baltes NJ, Gil-Humanes J, Voytas DF. Genome Engineering and Agriculture: Opportunities and Challenges. Progress in Molecular Biology and Translational Science. 2017; 149: 1-26. doi: 10.1016/bs.pmbts.2017.03.011.
[47] Miller K, Eggenberger AL, Lee K, Liu F, Kang M, Drent M. An Improved Biolistic Delivery and Analysis Method for Evaluation of DNA and CRISPR-Cas Delivery Efficacy in Plant Tissue. Scientific Reports. 2021; 11(1):7695. doi: 10.1038/s41598-021-86549-9.
[48] Murovec J, Guček K, Bohanec B, Avbelj M, Jerala R. DNA-Free Genome Editing of Brassica Oleracea and B. Rapa Protoplasts Using CRISPR-Cas9 Ribonucleoprotein Complexes. Frontiers in Plant Science. 2018; 9: 1594. doi: 10.3389/fpls.2018.01594.
[49] Zhang Y, Iaffaldano B, Qi Y. CRISPR Ribonucleoprotein-Mediated Genetic Engineering in Plants. Plant Communications. 2021; 2(2). doi: 10.1016/j.xplc.2021.100168.
[50] Mishra R, Joshi RK, Zhao K. Base Editing in Crops: Current Advances, Limitations and Future Implications. Plant Biotechnology Journal. 2020; 18(1): 20-31. doi: 10.1111/pbi.13225
[51] Eid A, Alshareef S, Mahfouz MM. CRISPR Base Editors: Genome Editing Without Double-Stranded Breaks.

DOI: https://doi.org/10.54393/df.v6i1.120

Biochemical Journal. 2018; 475(11): 1955-1964. doi: 10.1042/BCJ20170793.

[52] Cheng H, Hao M, Ding B, Mei D, Wang W, Wang H, *et al.* Base Editing with High Efficiency in Allotetraploid Oilseed Rape by A3A-PBE System. Plant Biotechnology Journal. 2021; 19(1): 87-97. doi: 10.1111/pbi.13444.

[53] Butt H, Rao GS, Sedeek K, Aman R, Kamel R, Mahfouz M. Engineering Herbicide Resistance via Prime Editing in Rice. Plant Biotechnology Journal. 2020; 18(12): 2370. doi: 10.1111/pbi.13399.

[54] Hirsch F, Iphofen R, Koporc Z. Ethics Assessment in Research Proposals Adopting CRISPR Technology. Biochemia Medica. 2019; 29(2): 206-213. doi: 10.11613/BM.2019.020202.

[55] Cathomen T, Schüle S, Schüßler-Lenz M, Abou-El-Enein M. The Human Genome Editing Race: Loosening Regulatory Standards for Commercial Advantage? Trends in Biotechnology. 2019; 37(2): 120-123. doi: 10.1016/j.tibtech.2018.06.005.

[56] Greene M and Master Z. Ethical Issues of Using CRISPR Technologies for Research on Military Enhancement. Journal of Bioethical Inquiry. 2018; 15(3): 327-335. doi: 10.1007/s11673-018-9865-6.

[57] Hundleby PA and Harwood WA. Impacts of the EU GMO Regulatory Framework for Plant Genome Editing. Food and Energy Security. 2019; 8(2): e00161. doi: 10.1002/fes3.161.

[58] Shinwari ZK, Tanveer F, Khalil AT. Ethical Issues Regarding CRISPR Mediated Genome Editing. Current Issues in Molecular Biology. 2018; 26(1): 103-110. doi: 10.21775/cimb.026.103.