A seminar paper

on

Advances in Genome Editing for Crop Improvement

Submitted to

Course Instructors	Major Professor
Dr. A. K. M. Aminul Islam Professor, Dept. of Genetics and Plant Breeding, BSMRAU	**Dr. Md. Tofazzal Islam** Professor, Institute of Biotechnology and Genetic Engineering (IBGE), BSMRAU
Dr. Satya Ranjan Saha Professor, Department of Agroforestry and Environment, BSMRAU	
Dr. Shaikh Shamim Hasan Professor, Dept. of Agricultural Extension and Rural Development, BSMRAU	
Dr. Dinesh Chandra Shaha Associate Professor, Dept. of Fisheries Management. BSMRAU	

Submitted by

Rojana Binte Azad

Reg. no. 17-05-4248

MS student

Institute of Biotechnology and Genetic Engineering (IBGE),

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BANGABANDHU SHEIKH MUJIBUR RAHMAN AGRICULTURAL UNIVERSITY, GAZIPUR- 1706

Advances in Genome Editing for Crop Improvement[1]

By

Rojana Binte Azad[2]

ABSTRACT

The advent of genome editing has generated a great deal of excitement, especially among agricultural scientists, due to its simplicity and ability to provide new opportunities for the development of improved crop varieties with the precise addition of beneficial traits or removal of undesirable traits. From recombinant DNA technology to engineered endonuclease, genome editing has come in a long way. Among the genome editing tools Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-associated protein (CRISPR/cas) has shown greater promise in crop genome improvement. Application of CRISPR/Cas in editing the crop plant genome has shown strategies for the increasing yield, quality, domestication and pest/stress tolerance. More advance CRISPR related technologies like base editing, prime editing, are emerging with more precision to fill up the drawbacks. Also good number of genome edited crops like (Gamma-aminobutyric acid (GABA) tomato, button mushroom and soybean have received access as non-genetically modified organism (non-GMO) for cultivation in the USA and Japan, also many countries including EU are considering on it.

Keywords: Genome editing, Crop improvement, CRISPR/Cas, Genome edited crops, Food security

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[2]MS student, Institute of Biotechnology and Genetic Engineering (IBGE), BSMRAU, Gazipur-1706

TABLE OF CONTENTS

Chapter	Title	Page No.
	ABSTRACT	I
	TABLE OF CONTENTS	II
	LIST OF TABLES	III
	LIST OF FIGURES	IV
I	INTRODUCTION	1-2
II	MATERIALS AND METHODS	3
III	REVIEW OF FINDINGS	4-22
IV	CONCLUSIONS	23
	REFERENCES	24-25

LIST OF TABLES

Table No.	Title	Page No.
1	Comparative inside performance of Genome editing tools of Genome editing tools	8
2	CRISPR edited some major crops with improved qualities/traits	16-18

LIST OF FIGURES

Figure No.	Title	Page No.
1	Different genome editing tools/ site directed endonucleases	6
2	Pathways of DNA double strand break repair mechanism	7
3	Processes of genome editing by different SDNs	8
4	Thriving of CRISPR research on plants throughout the years	10
5	CRISPR edited crops and traits	10
6	Past, present and Future of CRISPR	11
7	Domestication of wild tomato	12
8	Domestication of Wild rice	12
9	Development in plant breeding techniques	13
10	Resistance of wheat against Powdery mildew	14
11	Resistance of wheat against Powdery mildew without any yield penalties	14
12	CRISPR edited resistance rice against blast disease	15
13	Performance of CRISPR edited wheat	19
14	Examples of potential uses of base editing and prime editing in crop improvement	20
15	Transgene free genome editing in crop using mobile CRISPR element	21
16	Regulatory condition of Genome edited crops throughout the world	22

CHAPTER I

INTRODUCTION

Genome is the entire genetic information of an organism that consists of the nucleotide sequences of organism's DNA. Genome can be modified or altered artificially by some genome editing techniques. This techniques enable new insights into the functional genomics of organism like human, plant, and microbe. It also allows scientists to regulate the gene expression patterns in a pre-determined region. In this process the genetic materials (DNA, RNA) can be added, deleted, or replaced in a specific location of the genome. In 1972, Paul Berg and team revealed the molecular mechanism of recombinant DNA technology for the first time to the world and showed the path to change the heritable material (DNA) directly within the cell (Hanna, 1991). The molecular genetics and biochemistry of bacteria and viruses have been the subject of extensive research for many years. Utilizing knowledge from the studies numerous methods of manipulating DNA were developed to create transgenic microorganisms, plants and crop species. A lot of attention has been paid in developing new tools and applying them in the field of genetic engineering, which has sped up the entire procedure along. However, with time genome editing tools has come in long way. The engineered endonucleases like Mega-nucleases (homing endonuclease), Zinc Finger Nuclease (ZFN), Transcription Activator-Like Effector Nuclease (TALEN) and Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-associated protein CRISPR/Cas system are the pioneer tools in genome editing. In particular, CRISPR/Cas genome-editing technology has so far been shown the greatest promise in genome editing (Shan *et al*., 2013). Wining of Nobel prize by two discoverers of CRISPR as a genome editing tool, Jennifer Doudna and Emmanuelle Charpentier surely secures it's impact on biological research as well as on society.

The advent of genome editing has generated a great deal of excitement, especially among agricultural scientists, due to its simplicity and ability to provide new opportunities for the development of improved crop varieties with the precise addition of beneficial traits or removal of undesirable traits. Research is being done to develop crop types with high yield, more resilient to stress, stronger disease and insect resistance, less input requirement, and more nutritious. The world population rise along with climate change demand increase in crop production by 50% by 2030 and by 70–110% by 2050 for a well-fed world population (Godfrey *et al*., 2010). As

alternative approaches to conventional methods of crop production recently developed genome-editing toolkits are economically feasible, rapid and non-GMO solutions for precisely modifying plant traits to the desired genotype or crop variety for improvement of resistance to stresses and yield (Islam, 2019). Recent studies have shown that CRISPR/Cas genome-editing technology is potential for precise modification of plant genome for improvement of useful traits such as yield, quality and stress tolerance of major food crops such as rice, maize and wheat (Haque *et al*., 2018). Recent success and advances of genome editing tools has exploded the interest. Also GE is a precise modification that has no or minor changes and there is no external gene insertion like in genetically modified (GM) organisms. Instead, the target genes are identified, cut, and modified in very precise ways. So there has been a great deal of interest in the safety issues concerning the foods modified using GE technology.

Considering all of the factors, the objectives are follows:

1. to understand concept and mechanism of different genome editing tools
2. to explore accomplishment of genome editing tool CRISPR/cas in crop improvement
3. to elucidate challenges and opportunity in crop genome editing

CHAPTER II

MATERIALS AND METHODS

This seminar paper is completely a review article. So all information presented in this paper are collected from secondary sources. These sources consists of different articles published in different journals, online books, unpublished and published authenticate reports. After gathering all the information available, I agreed, organized, and explained in my way and finally prepared to meet the focused objectives.

Furthermore, various reliable information, good suggestions and kind of consideration from my honorable major professor and along with seminar lecturers, also provided many important suggestions for preparing this seminar paper. Subsequently, the collected information was organized systematically to present in this paper. Some information also presented in tabulated and graphical form.

CHAPTER III

REVIEW OF FINDINGS

3.1 Concept and mechanism behind the genome editing tools

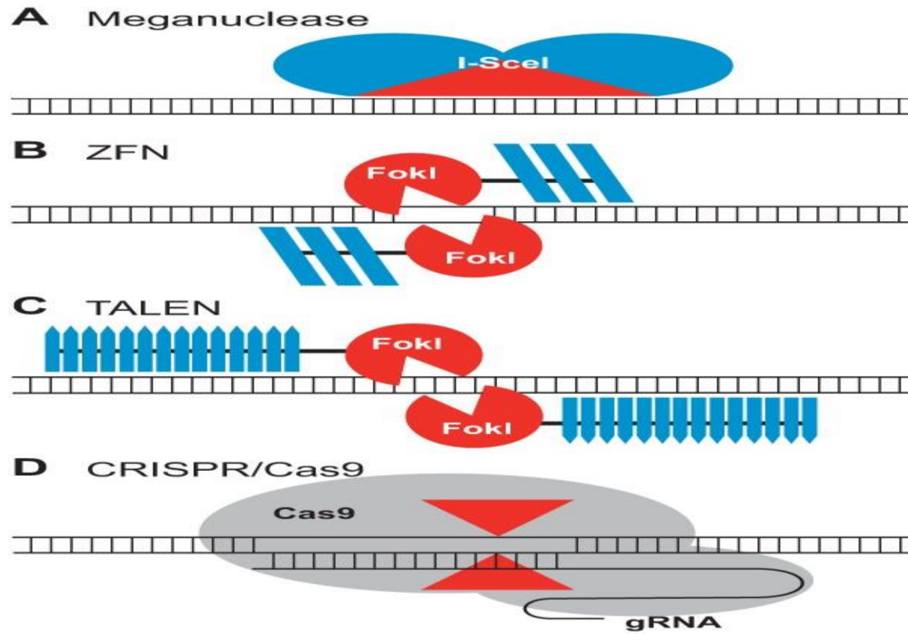
3.1.1 Genome editing tools

Genome editing uses site directed nucleases (SDNs) to make a desired change at the specific location(s) in the genome (DNA sequence) that may either be a small deletion, a substitution or the addition of a number of nucleotides or bases. It utilizes protein DNA interactions to target a specific location in the genome. The genome editing process includes two steps, 1) targeting a DNA site of interest in the genome and 2) subsequent editing.

There are four major types of SDNs or engineered endonuclease. They are 1) Meganuclease (Homing endonuclease), 2) Zinc Finger Nuclease, 3) Transcription Activator-Like Effector Nuclease (TALEN) and 4) Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-associated protein (CRISPR/cas). Meganucleases and Zinc Finger Nucleases (ZFNs) created the foundation for the engineered endonuclease back in the 1990s. Meganucleases (MegaN) are endonucleases found naturally in prokaryotes, archaea, and unicellular eukaryotes which can identify and remove extensive DNA sequences (from 12 to 40 base pairs) that are unique or nearly identical in the large portion of genomes (Gallagher *et al*., 2014). They are also able to change their recognition sequence through protein engineering, which allows them to replace, remove, or modify any sequence of interest in a very effective and focused manner. They have a larger recognition site than the restriction enzymes used in recombinant DNA technologies (Paques & Duchateau, 2007). On the other hand, Zinc-finger nuclease (ZFNs) are considered as one of the most efficient and effective tools of first generation genome editing. After the discovery of the functional Cys2-His2 zinc-finger domain, chimerically engineered nucleases ZFNs was developed. The structural makeup of ZFNs comprises mostly of two domains: (1) The DNA-binding domain, which has 300–600 zinc-finger repeats which is capable of monitoring and reading between 9 and 18 base pairs (bp) for each zinc-finger repeat (Carlson *et al.*, 2012). (2) the DNA cleavage domain, also known as the nonspecific cleavage domain of the type II restriction endonuclease Fok1 and acting as the DNA cleavage domain in ZFNs (Carroll *et al*., 2011). Then during the extensive use of these engineered endonucleases started to show some drawbacks. So

scientist keep searching more alternatives and then a more sophisticated version of ZFN was launched in 2010. It was Transcription Activator-Like Effector Nuclease (TALEN) that is easier and sophisticated than the previous strategies. TALENs is considered as substitution of ZFNs. Like ZFNs, TALENS are chimeric nucleases formed by coupling of 13–28 transcriptional activator-like effector (TALE) repeats, the DNA binding domain with FokI endonuclease, the cleavage domain (Becker & Boch, 2021). TALEN technology first introduced commercially available genome edited crop soybean in 2019 [http://www.calyxt.com/].

In the year of 2012, based on the principle of prokaryotic immune system, scientists proposed a genome editing tool called CRISPR. CRISPR was first reported in prokaryotes as an immune mechanism to fight against invading viral and plasmid DNA (Makarova *et al*., 2011). Eventually with time it has gained much popularity. Further simplification in its use, enables laboratories worldwide to adopt genome editing as a routine technology. Compared with other SDNs, the CRISPR/Cas systems are more efficient and straightforward for genome editing because the specificity of editing is determined by nucleotide complementarity of the guide RNA to a specific sequence without complex protein engineering. It consists of two core components: the guide RNA (gRNA or sgRNA) and the Cas9 protein. The gRNA constitutes CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA). The former contains a ~20 nt fragment (also known as a spacer, complementary to a specific site of target genes), followed by a protospacer adjacent motif (PAM) in the target genes of interest. Under the guidance of gRNA, Cas9 nuclease creates DSBs at ~3 bp upstream of the PAM motif (Jinek *et al*., 2012). The discovery and engineering of CRISPR has simplified the process of rapidly and efficiently targeting protein domains in areas of interest in a genome (Doudna and Sternberg, 2017).

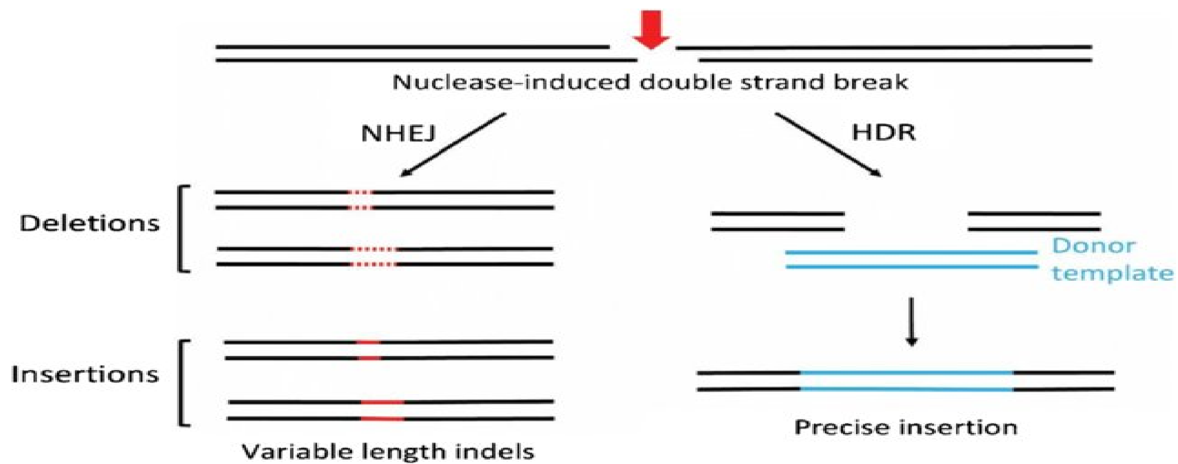


(Source: (Voytas & Gao, 2014)

Figure 1: Different genome editing tools/ site directed endonucleases

3.1.2 DNA double strand break (DSB) repair mechanism

Site-directed nuclease (SDN) genome editing involves the use of different DNA cleavage enzymes (nucleases) that cut DNA at specific locations using various DNA binding systems. After the DNA double strand break (DSB), cellular DNA repair mechanisms recognize the cut and repair the damage, using one of two pathways that are naturally present in cells. Two main mechanisms of DNA repair, Homologous-Direct Repair (HDR) or Non-Homologous End-Joining (NHEJ) path, are followed that allow deletion, addition or replacement of the nucleotide sequences. The cleavage repaired in NHEJ way, usually results in gene knockout or loss of protein function. Alternatively, when an exogenous DNA repair template is provided, HDR can be triggered, resulting in the introduction of the repair template into a target genomic region (Symington & Gautier, 2011).



(Source: Yamamoto & Gerbi, 2018)

Figure 2: Pathways of DNA double strand break repair mechanism

3.1.3 Process of genome editing

Depending on the nature of the edit that is carried out, the process is divided into three categories: SDN 1, SDN 2 and SDN 3. SDN-1 relies on the endogenous capacity to repair breaks in DNA. Insertions and deletions around the cut site change protein synthesis mechanisms so that a targeted protein can be knocked out and its expression terminated. SDN-2 uses a foreign donor nucleic acid template to perform a precise edit at the cut site, which is incorporated into the host genome. The process is inefficient however and the result is often the same as for the SDN-1 protocol. SDN-3 also relies on foreign donor DNA that is inserted into the cut site. However, unlike SDN-2, which elicits small, precise changes, SDN-3 can insert large fragments of DNA, including entire genes. SDN-3 is also inefficient depending on circumstance. Fundamentally SDN-1, SDN-2 and SDN-3 respectively effect DNA disruption, DNA correction and DNA insertion (Sprink *et al*., 2016).

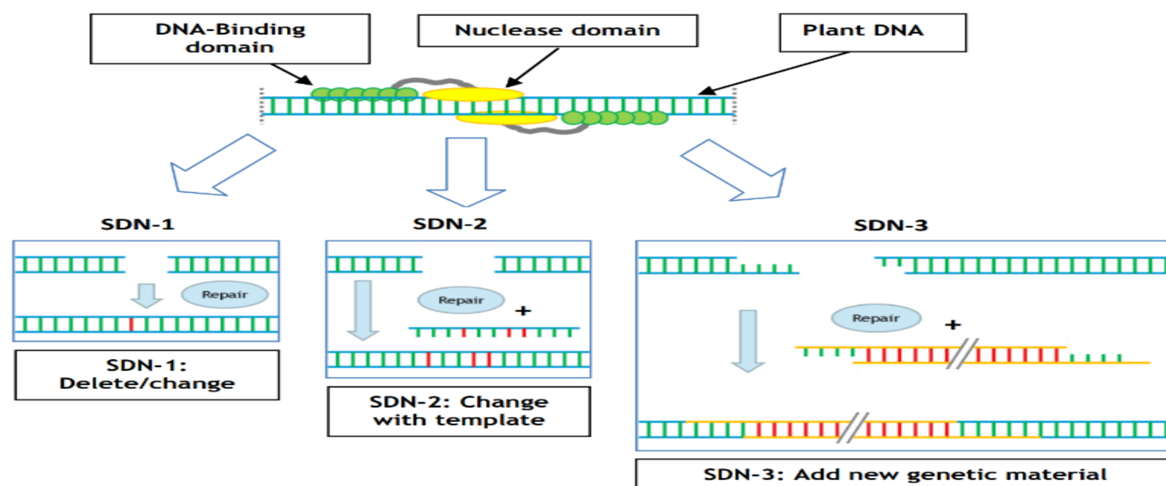


Figure 3: Processes of genome editing by different SDNs

Although all genome editing tools have same DSB mechanism, based on specification, target site range and cleavage specificity there are some differences.

Table 1: Comparative inside performance of Genome editing tools

Features	Meganuclease	ZFN	TALEN	CRISPR
Target range	12-40 bp	18-24 bp	24-59 bp	20-22 bp
Target recognition specificity	Higher	Higher	Higher	Higher
Target specificity domain	MN domain	Zinc finger domain	TALE domain	sgRNA
Target range	Unlimited	Unlimited	Unlimited	limited by PAM
Cleavage specificity	High	High	High	High with multiplexing capacity
Mismatch tolerance at target site	Moderate	Moderate	Low	Higher
Cleavage domain	MN nuclease domain	Fok 1 nuclease domain	Fok 1 nuclease domain	Cas protein
Repair mechanism	NHEJ/HDR	NHEJ/HDR	NHEJ/HDR	NHEJ/HDR

Multiplexing	Unattainable	Unattainable	Difficult to obtain	High multiplexing capacity
Off target effect	Comparative much off target effects	Few off target effects	Unavoidable off target effect	Moderate off targets, but can be minimized
Construction	Technically difficult and cumbersome	Challenging and complex technique	Comparatively easier	Quick and easy procedure

From the comparative study it is clear that the CRISPR/Cas technology is faster, cheaper, precise and highly efficient in editing genomes even at the multiplex level. The main advantage of CRISPR/Cas9 over the other technologies is the further simplification in its use. In fact, the original implementation of ZFN and TALEN site-directed nucleases for genome editing has proven to be cumber-some as it requires sophisticated protein design, synthesis and validation (Islam, 2019).

3.2 Accomplishment of CRISPR/Cas in crop genome editing

3.2.1 Current status of CRISPR as genome editing tool

After the introduction of CRISPR-Cas9 as a genome-editing technology in 2012, the CRISPR toolbox and its applications have profoundly changed basic and applied biological research. CRISPR has made revolution in crop genome editing and most of the studies on genome editing have been performed using a CRISPR/Cas-System. Research publications on the application of CRISPR in planta have thrived throughout these years. Among the major crop plants, rice occupies the first position in CRISPR related research for crop improvement followed by maize, wheat, tomato and soybean. While in traits improvement research by CRISPR agronomic traits and biotic stress tolerance are given more emphasis.

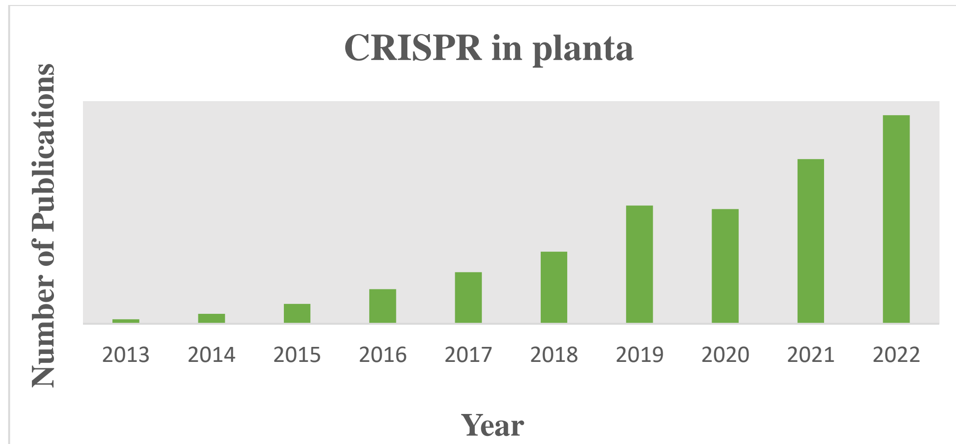


Figure 4: Thriving of CRISPR research on plants throughout the years

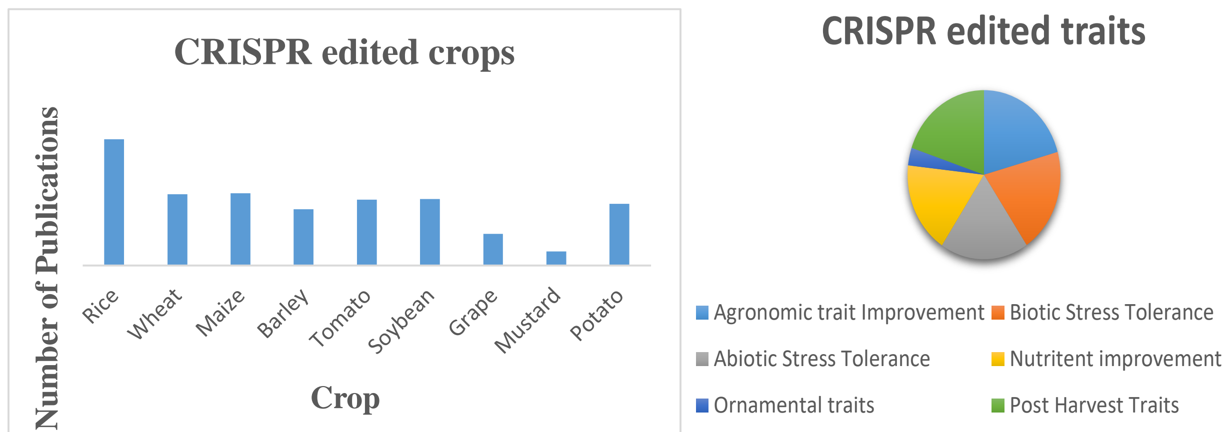
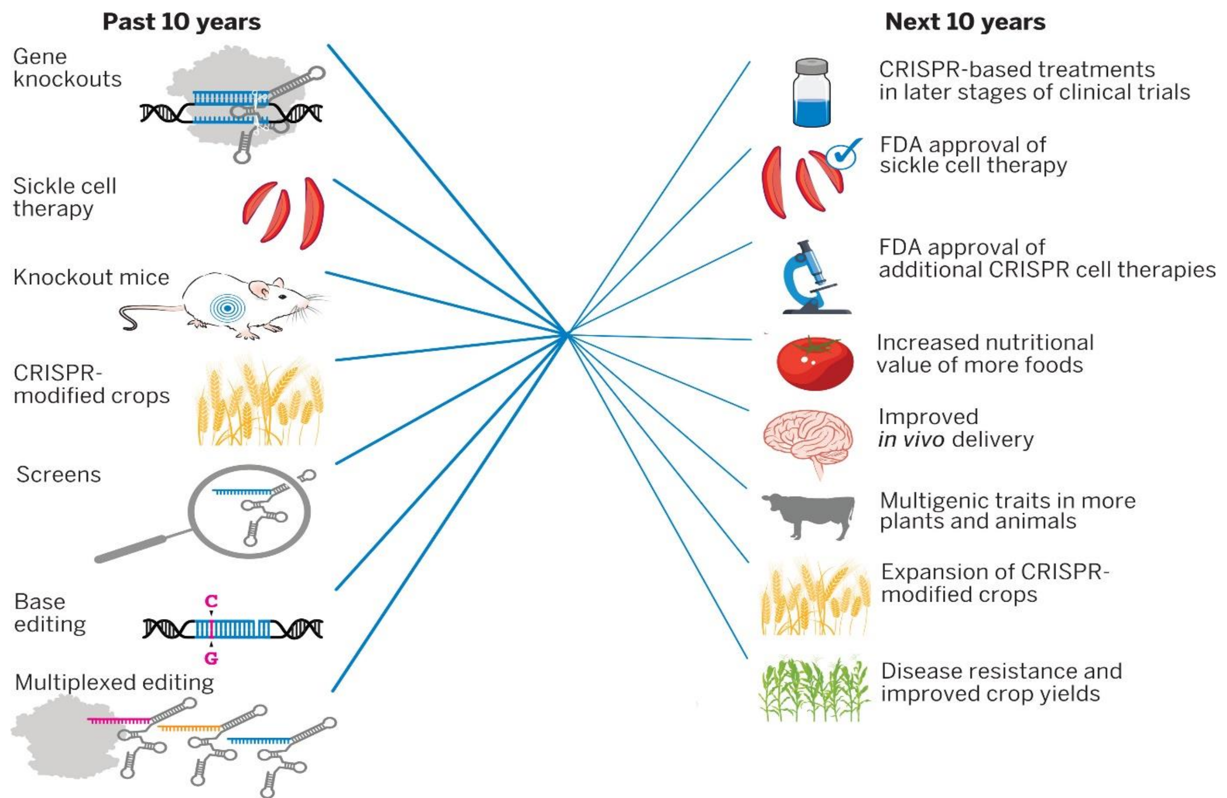


Figure 5: CRISPR edited crops and traits

On the occasion of a decade of the publication of CRISPR-Cas9 as a genome-editing technology Wang and Doudna reviewed the journey of CRISPR and highlighted specific examples in medicine and agriculture that show how CRISPR is already affecting society, with exciting opportunities for the future (Wang & Doudna, 2023). CRISPR modification for Crop domestication, increased nutrients value, improve crop yield and disease resistance are the main achievements in agriculture.

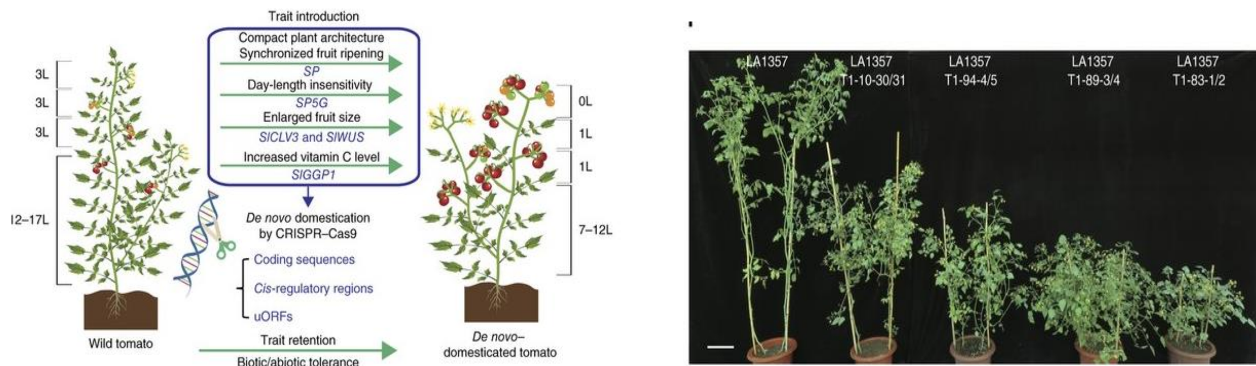


(Source: Wang & Doudna, 2023)

Figure 6: Past, present and future of CRISPR

3.2.1.1 Crop domestication

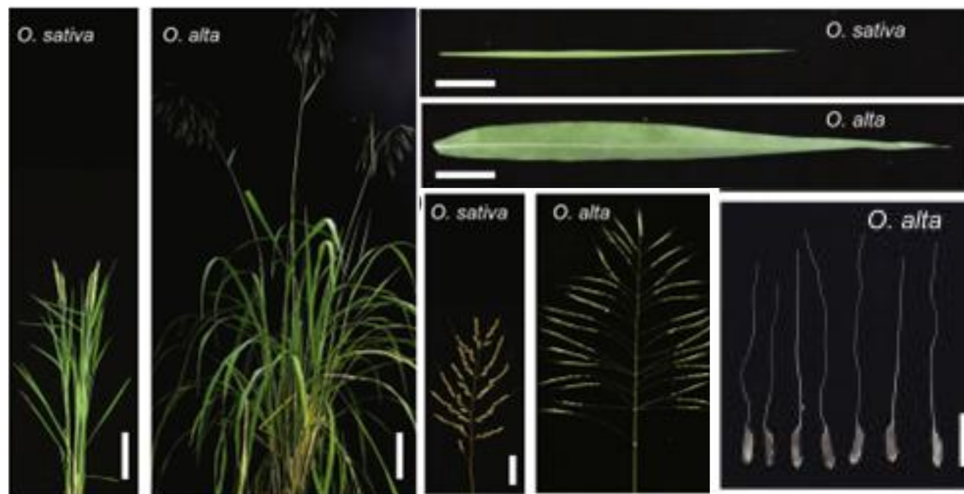
CRISPR/Cas has offered the possibility to domesticate novel species in a short time. Contrasting to traditional breeding methods, CRISPR-Cas technology provides a faster means to generate superior germplasm by deleting undesirable genetic factors that cause unwanted traits or introducing gain-of-function mutations through precise genome editing. CRISPR was employed in wild tomato for multiplex editing to introduce desire traits for field production. Newly developed variety is highly stress tolerance. In the wild type of tomato multiple editing was done. The representative plants showed compact plant architecture and some desirable agronomic traits (Li *et al*., 2018).



(Source: Li *et al*., 2018)

Figure 7: Domestication of wild tomato

A recent study described potential de novo domestication of wild rice to obtain new commercial rice varieties. Using multiplex gene editing, researchers improved six agronomic traits, including seed shattering, awn length, plant height, grain length, stem thickness and heading date in wild rice (Yu *et al*., 2021).



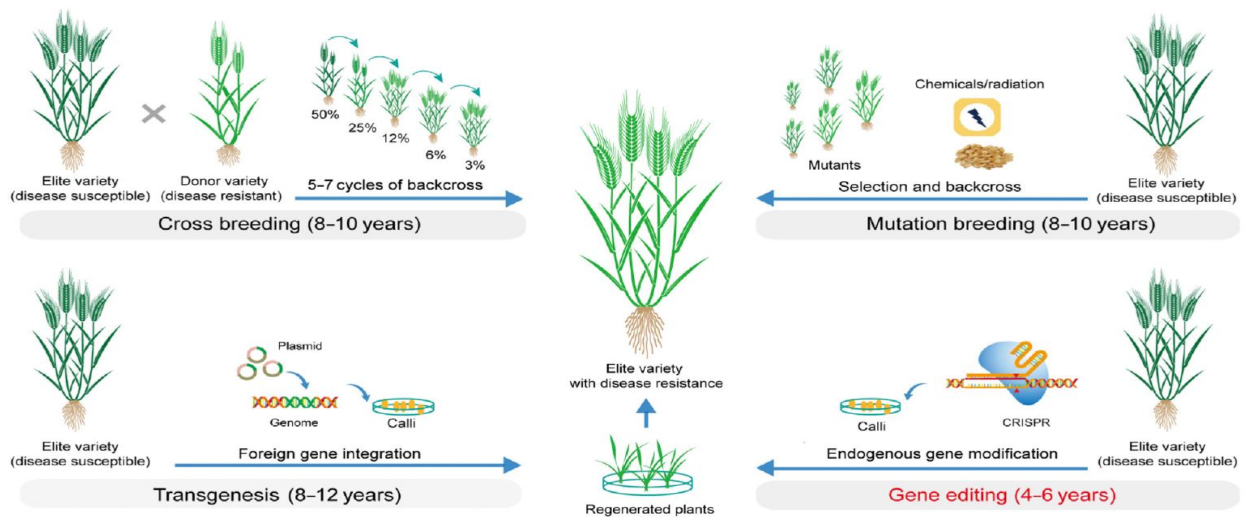
(Source: Yu *et al*., 2021)

Figure 8: Domestication of Wild rice

Still many currently important crops have not been exhaustively domesticated. Application of gene editing has been explored to increase the utility of orphan crops such as sorghum, millet, cowpea and quinoa (Gao, 2021).

3.2.2.2 Development in plant breeding

Gene-editing technologies represent the latest step towards increasing precision in breeding crops. It has shorten the duration of breeding new elite plants unlike other methods of breeding for example cross breeding, mutation breeding. Unlike transgenesis foreign genes need not necessarily be inserted into a host genome, there are protocols for removing foreign material under some circumstances. Technologies, including CRISPR-Cas, enable specific areas of a genome to be targeted precisely and cut. Insertions and deletions of genetic material at the cut site alter protein production, ultimately allowing a germline to be developed with desirable traits.



(Source: FAO, 2022)

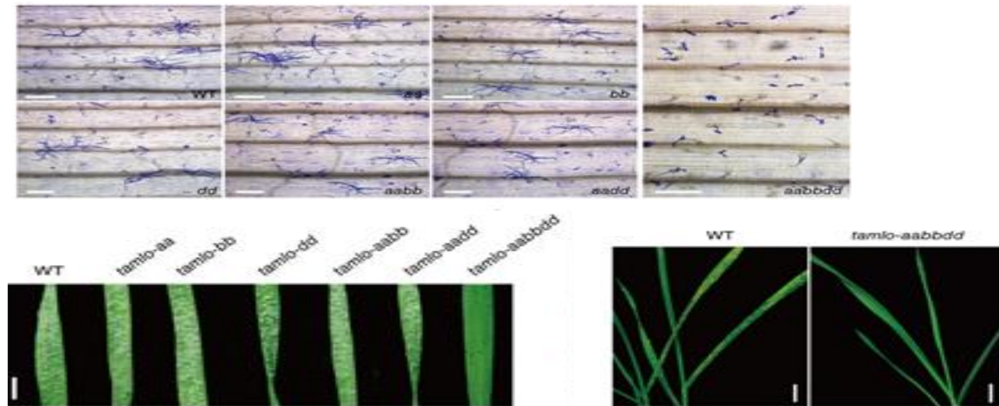
Figure 9: Development in plant breeding techniques

3.2.2.3 Disease resistance

Powdery mildew is considered as one of the major fungal diseases of wheat. A study demonstrated editing in wheat using CRISPR/Cas9 where copies of susceptible *MLO* gene were knocked out and the edited wheat developed heritable resistance against powdery mildew (Wang *et al*., 2014).

Seedling leaves of these mutants were challenged with conidiospores of a virulent pathogen race. Microscopic examination showed that the number of mildew microcolonies formed on the leaves was significantly reduced only in *tamlo-aabbdd* mutant plants. Also, no apparent fungal growth

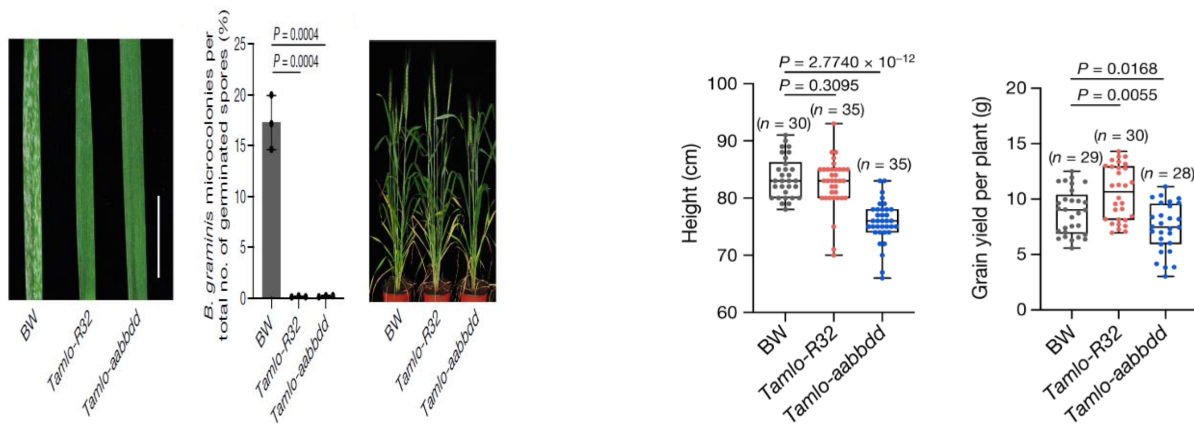
was observed on the leaves of the *tamlo-aabbdd* plants, although abundant fungal growth was found on the leaves of wild-type plants and those of the other mutant combinations. But this simple knock out of gene resulted in decrease crop yields.



(Source: Wang *et al*., 2014)

Figure 10: Resistance of wheat against Powdery mildew

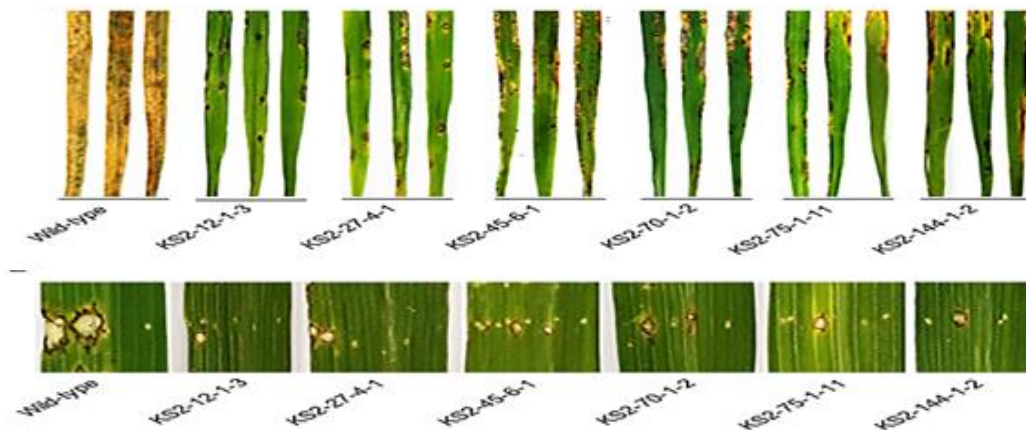
Subsequently another study demonstrated additional editing to stack genetic changes to rescue growth defects caused by the MLO knockout. Although the *Tamlo-aabbdd* and *Tamlo-R32* both mutant wheat plants were highly resistant to powdery mildew (*Blumeria graminis* f. sp. *tritici, Bgt*). But *Tamlo-R32* exhibited better result in both plant height and grain yield compared with wild-type plants. It was critical for developing high-yielding crop varieties with robust and durable disease resistance (Li *et al.,* 2022).



(Source: Li *et al*., 2022)

Figure 11: Resistance of wheat against Powdery mildew without any yield penalties

Another study reported the improvement of rice blast resistance by engineering a CRISPR/Cas9 SSN (C-ERF922) targeting the *OsERF922* gene in rice (Wang *et al*., 2016).



(Source: Wang *et al.,* 2016)

Figure 12: CRISPR edited resistance rice against blast disease

3.2.2.4 Crop quality improvement

Quality of crop such as nutritional contents, fragrance, color, size of grain etc. are involved in improvement of crop plants. The CRISPR/Cas genome-editing technology has success-fully been used in modifying crop plants for bringing the desired quality/traits. Several qualities/traits such as starch content, fragrance, nutritional value and storage quality in crops have been achieved by genome editing (Islam, 2019).

Table 2: CRISPR edited some major crops with improved qualities/traits

Crop	Target Gene(s)	Modified Traits
Rice	*BADH2*	Fragrant rice variety
	nif	Allows cereals to fix atmospheric nitrogen
	ALS, OsALS	Herbicide-tolerant rice
	OsCOLD1	Cold tolerance
	OsMODD, OsNAC14	Drought tolerance
	DEP1, PYL	Improvements in yield-related traits, such as dense and erect panicles and reduced plant height, improve productivity
	OsTT1	Thermo tolerance
	OsSIT1	Salt tolerance
	OsYSA and OsROC5	Albino-free rice seedlings
	GS3, GW2, GW5 and TGW6	Increased rice yield
	OsGSTU, OsAnP and OsMRP15	Decreased anthocyanin accumulation
	OsWaxy	Reduced synthesis of amylase (generation of glutinous rice)
	Gn1a, DEP1, GS3 and IPA1	Enhanced grain number, dense erect panicles and larger grain size
	SBEI and SBEIIb	High amylose rice
	TMSS	Thermo-sensitive genic male sterile rice
	AID	Herbicide-resistant rice
	LABA1	Barbless awns
	PROG1	Erect growth
	Rc	White pericarp
	Sh4	Non-shattering rachis
	Hd3a and RFT1	Breeding of early-maturing rice cultivars
	OsMATL	Haploid seed formation in Indica rice
	REC8, PAIR, OSD1 and MTL	Clonal propagation of hybrid rice through seeds
	BBM1	Asexual propagation through seeds
	qHMS7	Confers non-Mendelian inheritance in rice
	OgTPR1	Interspecific hybrid sterility in rice
	SaF/SaM	Overcame hybrid male sterility in rice
	P/TMS12-1 or LDMAR, TGMS	Development of photo-sensitive genic male sterile japonica rice lines,
	TGMS	Male sterile rice for hybrid technology
	OsARM1	Increased tolerance of rice to arsenic stress
	OsNramp5	Low Cd-accumulating Indica rice without compromising yield
	OsNRAMP5	Reduced uptake of cadmium from soil

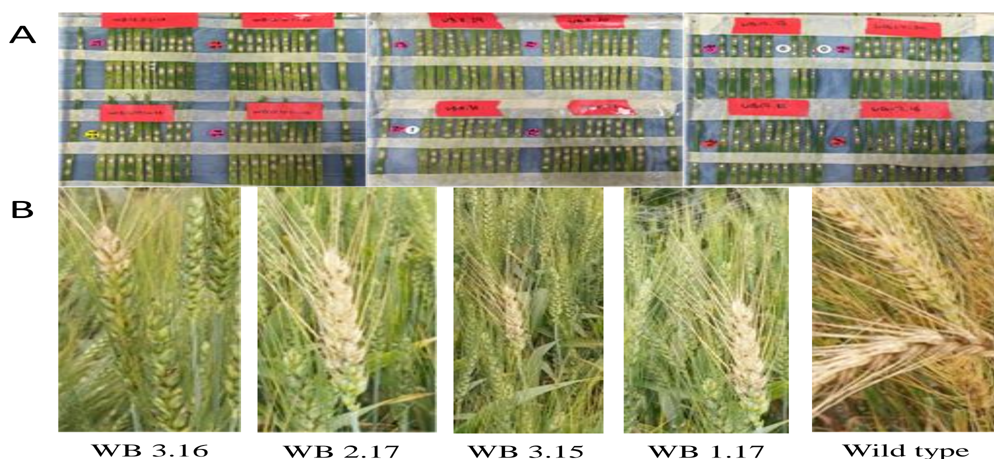
Crop	Target Gene(s)	Modified Traits
Wheat	*α- Gluten-encoding gene*	Low gluten wheat (reduces celiac diseasein human)
	Ms45	Rapid generation of male sterile line
	TaGW2-A1, -B1 and -D1	Controlled grain weight
	TaVRN1-A1	A to G base-edited wheat plants
	TaSBEIIa	High amylose wheat
	TaGASR7, GASR7	High the thousand kernel weight
Maize	*LIG, ALS2 and MS26 and MS45*	Resistant to chlorsulphuron when sprayed with the herbicide
	ZmTMS5	Generation of thermo-sensitive male-sterile maize
	ZmAgo18a, ZmAgo18b, a1 and a4	Anthocyanin biosynthesis
	tga1	Conferring naked kernels
	ARGOS	Improved maize grain yield under drought stress conditions
	LIG, ALS2, MS26 and MS45	Herbicide-resistant maize
Tomato	*DELLA or ETR1*	Increased tomato yield
	SlClV1, SlCLV2, SlCLV3, SlRRA3a	Increased fruit size
	ANT1	Modification of the tomato genome using geminivirus replicons
	CRTISO and PSY1	Gene targeting in tomato using geminiviral replicons
	CLV-WUS	Improved tomato yield
	SlSHR	Root development
	SP	Increased shelf life
	SlPDS, SlPIF4	Carotenoid biosynthesis
	FW2.2	Increased fruit weight
	SP5G	Rapid flowering and enhanced the compact growth habit of field tomatoes, resulting in a quick burst of flower production and early yield
	CycB	Increased lycopene accumulation
	RIN	Long shelf life
	Slagamous-like 9 SlIAA9	Parthenocarpy
	SGR1	Improved lycopene content in tomato fruit

Crop	Target Genes	Modified Traits
Soybean	*FAD2*	An effective DNA-free genome-editing tool for plant genome editing
	GmFEI2 and GmSHR	Improved root-specific trait
	Glyma04g36150, miR1509,miR1514, Glyma06g18790, GFP	Improved quality
	GmPDS11, GmPDS18	Carotenoid biosynthesis
	GmFT2a	Late flowering and increased vegetative growth
	DD20 and DD43	Herbicide-resistant soybean

(Source: adapted from Islam, 2019)

3.2.3 CRISPR/Cas in Bangladesh

Under a UK and Bangladesh collaboration project a total 7000 S-gene mutant lines of wheat against wheat blast fungus were developed using CRISPR/Cas technology. Both S-gene mutant lines were screened in both UK and IBGE, BSMRAU, Bangladesh lab and field. In detached leaf assay in growth room and in field some mutant plants showed moderate resistance against blast (KGF, 2021).



(Source: KGF, 2021)

Figure 13: Performance of CRISPR edited Wheat

A) Detached leaf assay on S-gene mutant wheat plants**. B)** S-gene mutant lines showed 32.67 to 39.33 % disease severity whereas wild type wheat exhibited 70.67 %.

Rapid detection of wheat blast pathogen using genome specific primers and CRISPR/Cas12a guide RNA technology also a landmark achievements (kang *et al,* 2021).

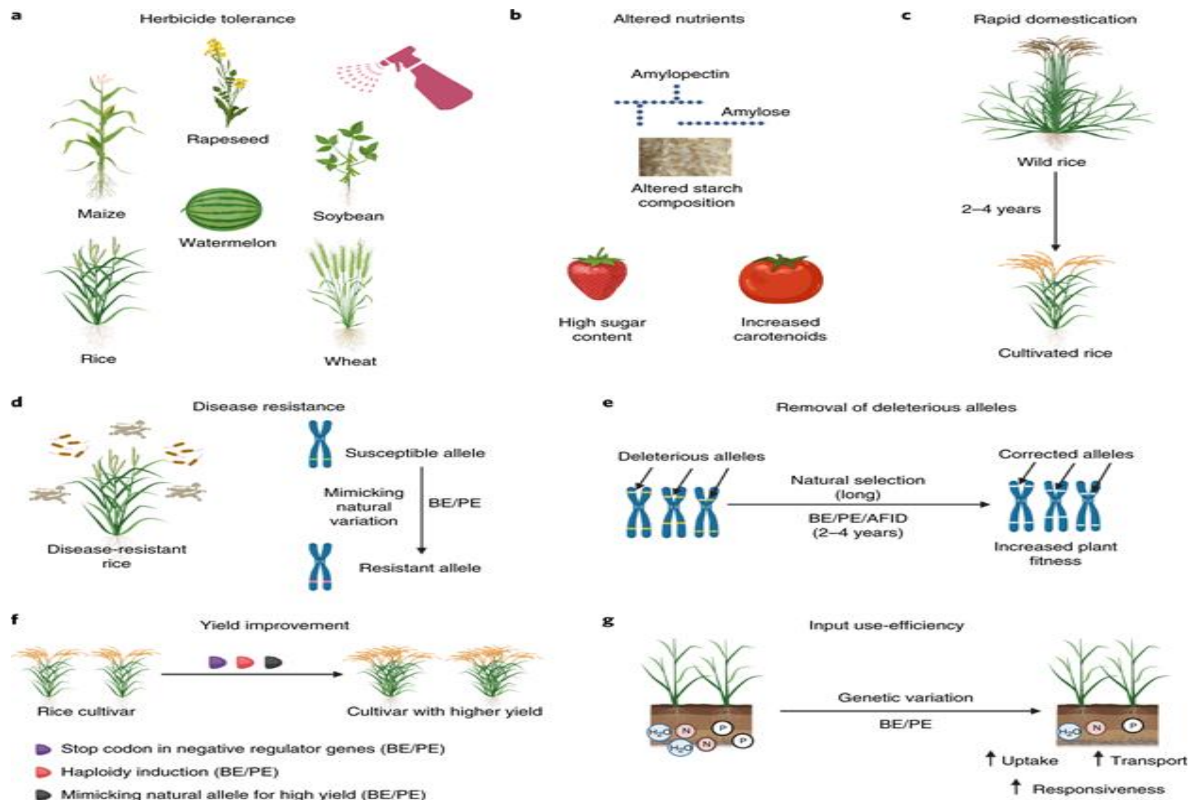
3.3 Challenges and Opportunity in crop genome editing

3.3.1 More precise editing without off target effects

The efficiency of genome editing obviously varies. Occasionally off-target editing happens instead of on-target editing. To reduce off target effects in CRISPR/Cas system, many alternatives of traditional Cas9 enzyme is present now. Several high-fidelity variants, such as eSpCas9(1.1), Cas9-HF1, HypaCas9, Cas9_R63A/Q768A, evoCas9, HiFi Cas9 and Sniper-Cas9 have been introduce to lower the off target effects (Kim et al., 2023).

In CRISPR/Cas system site directed nucleases can induce DNA double-stranded breaks followed by gene knockout by non-homologous end joining (NHEJ) repair or precise DNA sequence replacement by homology-directed repair (HDR). NHEJ-mediated mutagenesis is highly efficient in plants, it is typically used to generate gene knockouts and alteration. However, HDR is proven to be inefficient and limited precise genome editing in plants. As alternative there are newer, more precise, gene-editing technologies are available now. Such as base editing and prime editing technologies have recently been developed and demonstrated in plant species. These technologies are mainly based on Cas9 nickases that induce single strand break. Without any DSB in DNA, base editing and prime editing can introduce precise changes into the target genome at a single-base resolution (Molla *et al*., 2021).

Single-nucleotide variants and small indels have often been associated with mono and polygenic agronomic traits and are crucial for crop improvements. The base editing and prime editing technologies have numerous potential applications like, a) herbicide tolerance, b) altered nutrient, c) rapid domestication, d) disease resistance, e) crop fitness, f) yield improvement g) input use efficiency and so on.



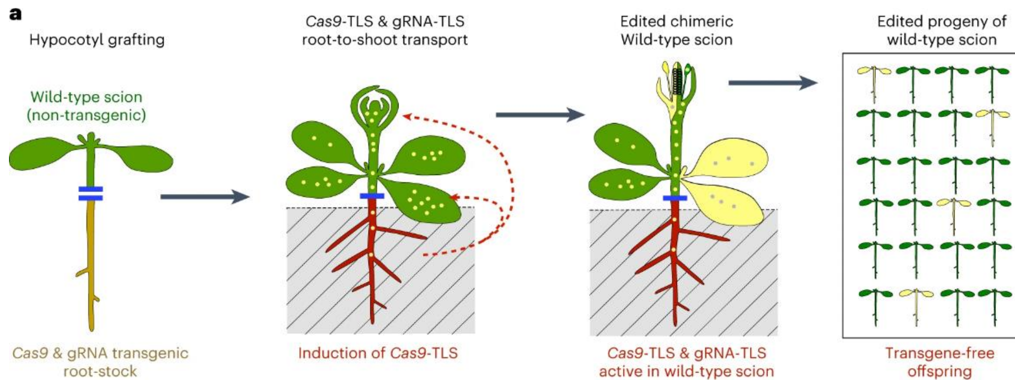
(Source: Molla *et al*., 2021)

Figure 14: Examples of potential uses of base editing and prime editing in crop improvement

3.3.2 Transgene free editing

The issue of 'transgene' is one of the most widely discussed in the field of genome editing. Emerging studies on novel genome editing tools are focused on transgene-free editing, which are deemed to be more 'regulatory-friendly' and may attract improved public approval. In genome editing process SDN1 and SDN2 do not include transgene. Generation of stable gene-edited plant lines using CRISPR/Cas requires a lengthy process to eliminate CRISPR/Cas9-associated sequences and produce transgene-free lines. Yang and the team have designed fusions of Cas9 and guide RNA transcripts to tRNA-like sequence motifs that move RNAs from transgenic rootstocks to grafted wild-type shoots (scions) and demonstrated heritable gene editing in wild-type *Arabidopsis thaliana* and *Brassica rapa*. The graft-mobile gene editing system enables the

production of transgene-free offspring in one generation without the need for transgene elimination, culture recovery and selection, or use of viral editing vectors (Yang *et al*., 2023).



(Source: Yang *et al.*, 2023)

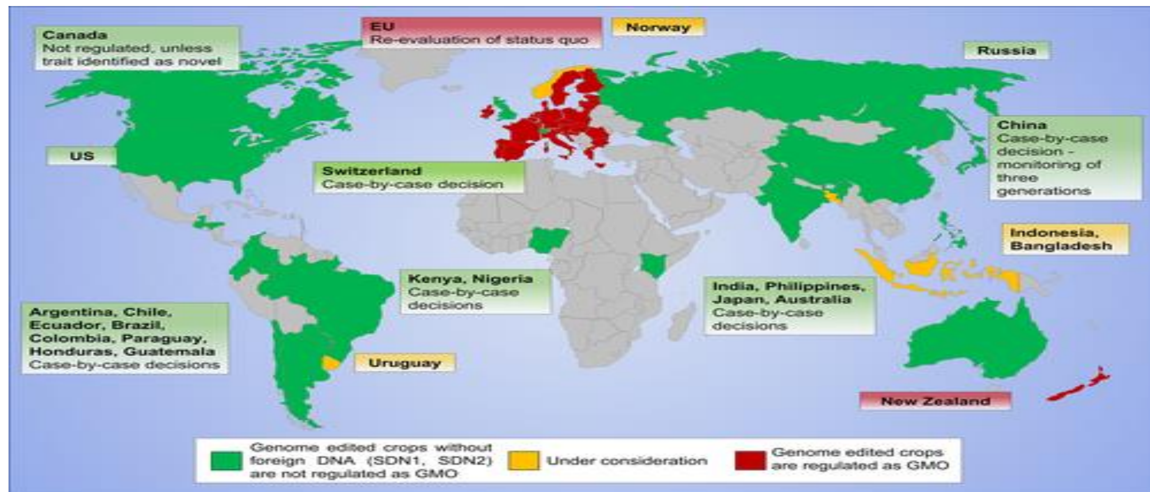
Figure 15: Transgene free genome editing in crop using mobile CRISPR element

The resulting offspring does not contain remnants of the scissors but retains the edited sequence. The resulting plant lines are thus not fundamentally different from lines that carry natural variants, and they do not contain any transgenes. It is anticipate that using graft-mobile editing systems for transgene-free plant production may be applied to a wide range of crop plants modifications.

3.3.3 Acceptance of genome edited crops

Large parts of the world have already introduced regulations or guidelines for crops that were subjected to genome editing. Over the past few years, many more countries have introduced guidelines that enable the use of such edited lines in agriculture in a similar way as conventionally bred lines as they do not contain a transgene (Buchholzer & Frommer, 2023). GABA tomato, button mushroom and soybean are the examples of commercially cultivated GE crops in Japan and USA respectively. An increasing number of adopter countries have exempted certain genome-edited (GE) crops from legal GMO pre-market approval and labelling requirements. Among them are major exporters of agricultural commodities such as United States, Canada, and Australia classified transgene-free, genome-edited lines as equivalent to conventionally bred lines. It remained open which path countries in continents with a high agricultural productivity, like Asia

and Africa, would take. However the non-adopter countries Japan, New Zealand, the EU, Norway, and Switzerland depend on import of large volumes of agricultural commodities from adopter countries. So, they are reconsidering their policies and legislations. Due to the relaxed legislation more GE plants are expected to enter the market soon (Spoke *et al*., 2022).



(Source: Buchholzer & Frommer, 2023)

Figure 16: Regulatory condition of Genome edited crops throughout the world

CHAPTER IV

CONCLUSION

Compared to other genome editing tools CRISPR/Cas technology has proven to be faster, precise, and highly efficient in editing genomes even at the multiplex level. It has become the most used genome editing tool in crops.

It's applications in crop for increasing yield, quality, domestication and pest/stress resistance have shown paths for future crop improvement strategies. Also recent developed two CRISPR related techniques, base editing and prime editing, have enhanced crop breeding opportunities. CRISPR has also started journey in Bangladesh like other countries, with a greater prospects in crop disease resistance and other traits improvement. So more optimization of CRISPR/Cas technology and wide application in all types of crops is needed to ensure the future food security.

Genome editing has excluded the concept of foreign genes. This has made genome edited crops more acceptable to people. So change in global regulatory system is a demand of time. It already can be said that GE is an effective mean for sustainable crop production. So, it is anticipated that the GE crops are going to fulfil the food demand of the future like as another green revolution.

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