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THE ENHANCEMENT OF PLANT DISEASE RESISTANCE USING CRISPR/Cas9 TECHNOLOGY

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Abstract—The technologies relating to genome editing have so far progressed rapidly as it has shown the importance of the implementation towards the build up of plan resistance towards pathogens infection. For the past recent years, multiple methods for site directed modification such as clustered regularly interspaced short palindrome repeats (CRISPR)/CRISPR-associated protein 9(cas9), zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Among the example methods, CRISPR/Cas9 has by far produced the best result due to the criteria it possessed such as higher success rate, easier design and implementation, less expensive and more versatile. This chapter will revolve around the CRISPR/Cas9 as how the methods will bring forth the advancement of plant protection against potential diseases. An achievement towards the viral diseases resistant on two main species (Arabidopsis and Nicotiana benthamiana) was highlighted to bring upon discussion. with the viral genome. The modification of crop species such as tomato, wheat, rice and citrus are some of the possible targeted CRISPR/Cas9 modifications.

Keywords— *CRISPR/Cas9, Crop improvement, Genome editing, Disease resistance, Virus*

1.1 Introduction

Selective breeding of plants method can be traced back to the earliest discovery at southern Mexico more than 6000-10,000 years ago. This shows that the genome editing concept has been already exploited and been used for the greater good among human history [1]. As the human population grows, the demand of crops is increasing and there must be hurdles to meet the demand. The advancement of the crop industry also comes with downsides due to the existence of fungi, viruses and bacteria since crops are highly affected by it, which then results in economic losses [2].

1.1.2 Interaction of plants and microorganism

Plant infection involves a complex interaction between a host plant and a pathogen, the defence mechanism may involve several components. Changes of interaction due to induced and natural mutation also impede certain processes in the mechanism of infection [3][4].

The early days of pre-genomic era, breeding techniques by identification of natural and mutant alleles with wanted criteria and incorporated into elite genotypes. The downside of this method is the unpredictable and imprecise where it produces the transfer of large genome regions instead of single gene insertions. However, throughout history, the method has been surprisingly successful as traditional crop breeding and has been used to give rise to new generation crops. Innumerable mutants have evolved by mutation induction to manifest enhanced defence to various diseases. Widely known mutants are induced at the mildew resistance locus (MLO) in barley for defence against powdery mildew [5], and mutations conferring resistance to several lettuce diseases [6]. The attraction of the MLO mutant is that the allele is not degraded and bestows unparalleled resistance towards mildew for 20 years [7]. The lastingness is because of the gene lockout. In other situations where defence against certain pathotypes is conferred by specific host gene allele, mutagenesis needs to be deployed to provide more precise single nucleotide mutations in the target gene sequence.

Discovery of genome and transcriptome sequences boosted the new era of plant breeding programs. Association genetics based on single nucleotide polymorphisms (SNPs) and other molecular markers are implemented in plant breeding, yielding important high throughput data for quantitative trait loci (QTL) identification. Large QTL was used in crops to provide pathogens with quantitative tolerance, coupled with the usage of global tolerance (R) genes inserted into varieties with superior agronomic characteristics.

New breeding techniques (NBTs) draw interest in plant science and include several diverse fields, for example developmental biology, abiotic pain tolerance or plant-pathogen resistance [8]. NBT provides the new and most effective biological methods to accurate genetic manipulation of single or several target genes. Research uses location-driven nucleases to insert double stranded breaks in DNA at specific locations. These breaks are restored through various pathways for the reconstruction of host cells, resulting either in minor insertions or deletions by close homologous end-joining (NHEJ) or microhomology-mediated end-joining (MMEJ), or in a transformed gene carrying programmed nucleotide changes copied from a reconstruction matrix by homologous recombination (HR). Meganucleases (MNs), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindrome repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) correspond to the four types of nucleases used in genome editing. The dramatic growth in articles documenting the usage of CRISPR / Cas9 highlights the reality that this technique needs fewer know-how and financial resources and has a higher rate of gene editing performance relative to the other nucleases available. The application of CRISPR / Cas9 editing has become a powerful tool for improving agronomic traits in crops in the future [9].

1.2 Biotechnology on CRISPR/Cas9

1.2.1 CRISPR/Cas9

CRISPR/Cas9 from *Streptococcus pyogenes* (SpCas9) has rapidly assumed an important role in various plant research applications and in many other areas [10]. A single-guide RNA (sgRNA) can bind to Cas9 in the CRISPR / Cas9 network and target it to different DNA sequences. A protospacer adjacent motif (PAM) condition restricts the potential target sequences inside a gene of interest. This limitation is of minor importance when the aim is simply to inactivate a gene at any position through targeted mutagenesis. It has much greater significance for genome editing directed at the exact alteration of different nucleotides within a gene.

Therefore, significant efforts are ongoing to identify Cas9-like proteins with specific PAM sequences, or to evolve the initial Cas9 from S.pyogenes to understand sequences in certain PAMs. For example, xCas9, an evolved version of SpCas9, has been shown to recognize a wide range of PAM sequences in mammalian cells including NG, GAA and GAT [11]. In plants, Cpf1 from Prevotella and Francisella with the PAM sequence TTTV, where "V" is A, C or G [12], is the most widely explored alternative to SpCas9. Also, Cpf1 is considerably smaller than Cas9, is capable of RNAse action to process its guide RNA and introduces a staggered double break that can be useful to improve homology-driven recombination and generate successful gene insertion.

1.2.2 Multiplex Genome Editing

One of the main benefits of CRISPR / Cas9 technologies with regard to MN, ZFN or TALEN is the simultaneous targeting of multiple genes with a single molecular structure [13]. In crops, numerous early reporting techniques for multiplex genome editing (MGE) were documented, all focused on a specific technique, i.e. the assembly of multiple

gRNAs under the guidance of a U3 or U6 promoter into a single build [14] [15] [16]. In maize the ISU Maize CRISPR platform requires up to four gRNAs to be cloned for targeting multiplex genes.

More recent multiplex systems harness the capacity of RNA molecules comprising tRNA sequences to self-clean themselves. Constructs alternating sgRNA and tRNA sequences under the influence of a single promoter U3 or U6 help to reduce the size of the construct and restrict the chance of silencing due to direct repetitions of promoter sequences. The use of such a strategy using polycistronic tRNA-gRNA (PTG) to produce genetic mutations in the genomes of TaLpx-1 and TaMLO was recorded in hexaploid wheat (Figure 1) [17].

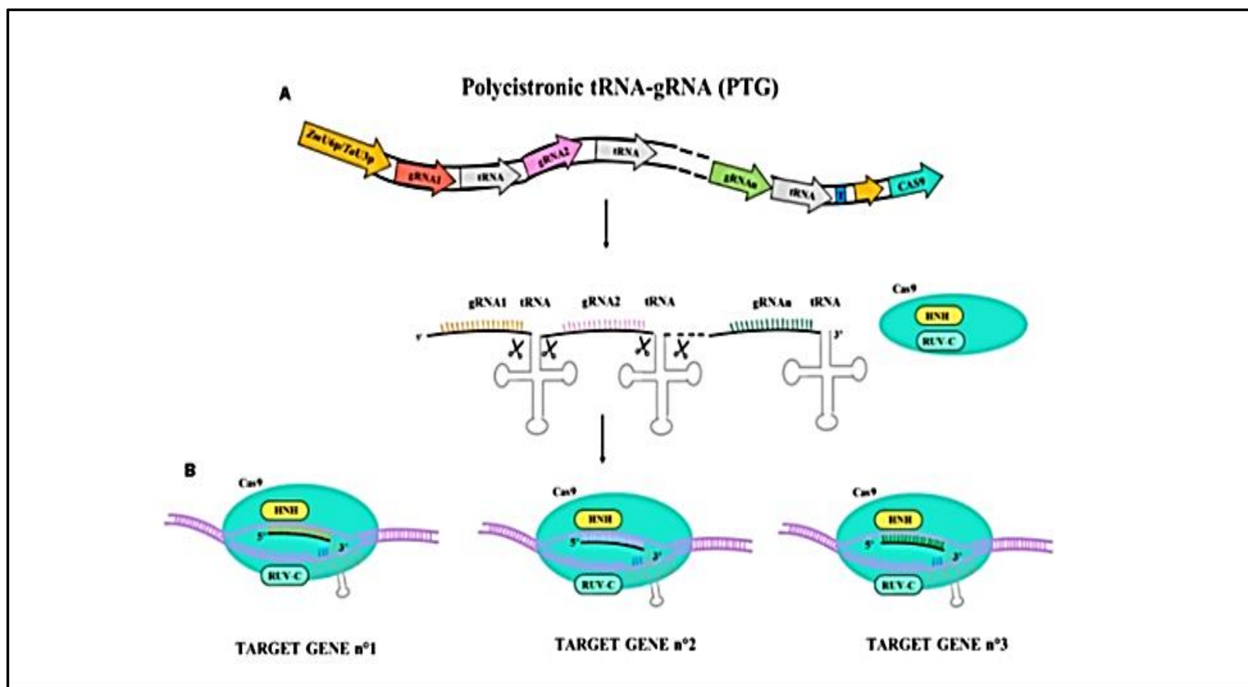

Figure 1: Illustrative diagram of polycistronic tRNA-gRNA (PTG) gene construct and targeting activity for Cas9. PTG is composed of t-RNA-gRNA repeats and is upregulated by ZmU6 promoter or TaU3 promoter according to the experimental design as different terminator regions (T) are adopted. (A) PTG primary transcript. Endogenous endonuclease cuts the tRNA ends and lets each tRNA-gRNA target the corresponding gene sequence. (B) In the PTG system more sequence targets are available (n° gene targets) and the different gRNA are represented in different colors (orange, pink, and green)[17]

1.2.3 Frequency and Limitation

High precision is also placed forward as a significant point in support of CRISPR / Cas9 technologies. As for example, when contrasted with mutagenesis caused by chemicals or irradiation. This poses the issue of to what degree a gRNA targets only entirely compatible genomic DNA sequences, and to what degree the CRISPR / Cas9 method may still identify and cleave certain genomic regions (off-target regions) and potentially inflict unwanted damage. Scientists and regulatory agencies invoke two forms of off-target effects: (i) the predicted off-target in high-sequence target-like genome regions, and (ii) the unintended off-target in unknown genome regions. The former is usually dealt with by amplifying PCR and sequencing regions considered to be close to the target, the latter by sequencing the entire genome [19].

Knowledge about the genome sequence is needed to determine anticipated off-target results. The analysis focuses on the 20 bp target sequence that includes base pairing with the gRNA but removes the 5′-NGG-3′ PAM. The PAM functions as a recognition site outside of the target element and does not give nuclease cleavage specificity [20]. The CRISPR / Cas9 framework often recognizes at least three malfunctions in the target sequence of 20 bp DNA. Most CRISPR / Cas9 design tools take this into account and only recommend unique gRNA designs which do not bind off-target theoretical sites with more than 17 bp identification anywhere in the genome. This state-of-the-art engineering becomes effortless if the gene

becomes special to the genome, but when the gene has one or more parallels, it is very complicated. That also implies that, without recent duplications, the design is usually simpler for diploid genomes than for recently duplicated or polyploid genomes. In silico genome analysis of possible target sequences in dicots and monocots, it has been reported that larger genomes produce, as predicted, more PAMs and more potential targets [21]. High accuracy varying from 87.3% to 94.3% was found in fairly clear genomes of Arabidopsis, corn, tomato, and soybean, while maize, a new allotetraploid with high rates of repetitive DNA, only reported 29.5% precise targeting [21].

Recent investigations have screened CRISPR / Cas9 knockout progenies in polyploid species to clarify the off-targeting problem in crops. An analysis of the CRISPR mutation incidence and mutation heritability of the allohexaploid wheat genes TaGW2, TaLpx-1, and TaMLO [17]. The findings for the three genes were different: strongly conserved for TaGW2 (the specific target sequence for all three genomes), mild for TaLpx-1 (the specific target sequence in two genomes), and weak for TaMLO. The study showed the CRISPR / Cas9 technology's flexibility in implementing complex gene editing where most genes have more than three homologous copies. The genome editing mechanism has also been studied across generations: new mutant variants have been recovered through several genome targets indicating the CRISPR / Cas9 transgenerational operation [17].

On OsBEIIb and OSBEIIa paralogs, another research on goal accuracy and productivity was performed in rice. The analysis shows the difference in gRNA prediction and mutagenesis performance, suggesting that gRNA will attain high mutation frequency with low expected output even as the forecast indicated specific goals with high mutagenesis scores [22]. Empirical research may seem appropriate to prevent putative inefficiency of gRNA. In addition, the authors also studied off-target mutagenesis and observed no mutation in the OSBEIIa paralog when only OsBEIIb was targeted, supporting the strategy's high precision.

To conclude, the CRISPR / Cas9 complex is able to bind one to three mismatches with lower efficiency sequences. Expected off-target mutations often occur but can be avoided by the CRISPR / Cas9 method being rigorously developed. Unexpected off-target mutations do not arise at a level greater than the plants' random mutation rate.

1.2.4 Strategies on Resistant Build Up

Advances of gene editing tools also opened up new avenues to boost crop resistance. The CRISPR / Cas framework has been used in recent years to respond to many agricultural challenges, including achieving improved tolerance to biotic stress [23]. The use of CRISPR / Cas methods was mainly studied against contamination by viruses, accompanied by attempts to enhance tolerance to fungal and bacterial diseases. Latest studies demonstrating the ability of the CRISPR / Cas technologies to create resistance to these pathogen groups will be addressed further below.

In certain economically important staple and specialty crops, plant viruses serve a serious threat. They are classified into six main classes depending on their genome nature: double-stranded DNA (dsDNA) viruses with no plant viruses in this category, single-stranded DNA (ssDNA), reverse-transcribing viruses, double-stranded RNA (dsRNA), negative sense single-stranded RNA (ssRNA-), and positive sense single-stranded RNA (ssRNA+) viruses[24]. Most research involving CRISPR-edited virus and fungal resistance plants have been based on the ssDNA geminivirus genomes as tabulated in Table 1(a) and (b).

1.3 Advantages of CRISPR/Cas9

As the times change rapidly, so do the changes in technologies that are becoming more sophisticated and current. For CRISPR technology, there are many benefits and lots of advantages due to the fact that this technology can be used to edit genes and will likely change the world with its own customization method. The purpose of CRISPR is very easy as it is a way to locate a particular bit of DNA inside a cell and then the next step in the editing of CRISPR genes is typically to change that piece of DNA.

For the first, clustered regularly interspaced short palindromic repeats (CRISPR). By using this CRISPR technology, it helps to solve a problem as quickly as possible in the agricultural sector. For 25 years agricultural scientists have been improving plants by biotechnology by moving genes from one species of plants or bacteria to another. Advances of gene editing tools also opened up new ways to improve plant

Table 1(a) : Resistance towards virus

Plant Species	Virus	Target Gene	Gene Function	Strategy	Reference
Nicotiana benthamiana and *Arabidopsis thaliana*	BeYDV	CP, Rep and IR	RCA mechanism	*Agrobacterium* – mediated transformation of leaves with Cas9/gRNA expression plasmid vectors.	[23]
Nicotiana benthamiana	BSCTV	LIR and Rep/RepA	RCA mechanism	*Agrobacterium* – mediated transformation of leaves with Cas9/gRNA expression plasmid vectors.	[24]
Nicotiana benthamiana	TYLCV BCTV MeMV	CP, Rep and IR	RCA mechanism	*Agrobacterium* – mediated transformation of leaves with a TRV vector in Cas9 overexpressing plants.	[25]
Nicotiana benthamiana	CLCuKoV MeMV TYLCV	CP, Rep and IR	RCA mechanism	*Agrobacterium* – mediated transformation of leaves with a TRV vector in Cas9 overexpressing plants.	[25]

Table 1(b) : Resistance towards ungal

Plant Species	Fungal	Target Gene	Gene Function	Strategy	Reference
Triticum aestivum	Powdery mildew (*Blumeria graminis f. sp. tritici*)	MLO-A1	Susceptibility (S) gene involved in powdery mildew disease.	Particle bombardment of immature wheat embryos with Cas9/gRNA expression plasmid vectors.	[15]
Solanum hycopersicum	Powdery mildew (*Oidium neolycopers ici*)	MLO-1	Major responsible for powdery mildew vulnerability.	*Agrobacterium* – mediated transformation of cotyledons with Cas9/gRNA expression plasmid vectors.	[21]
Vitis vinifera	Powdery mildew (*Erysiphe necator*)	MLO-7	Susceptibility (S) gene involved in powdery mildew disease.	PEG – mediated protoplast transformation with CRISPR ribonucleusproteins.	[19]
Vitis vinifera	Powdery mildew (*Botrytis cinerea*)	WRKY52	Transcription factor involved in response to biotic stress.	*Agrobacterium* – mediated transformation of proembryonal masses with Cas9/gRNA expression binary vectors.	[15]

resistance. The CRISPR system has been employed in recent years to respond to several agricultural challenges, including achieving improved resistance to biotic stress [28]. CRISPR/ Cas9 main advantages over other genome editing technologies are potentially its flexibility and performance. Since it can be used directly in bryos, CRISPR/ cas9 decreases the time taken to change target genes relative to gene targeting approaches focused on the use of embryonic stem (ES) cells. These DNA targeting proteins can be fused with various enzymatic activities following the deactivation of the CRISPR cas9 and cpf1 nucleus domains, which are distinct from the DNA recognition domain.

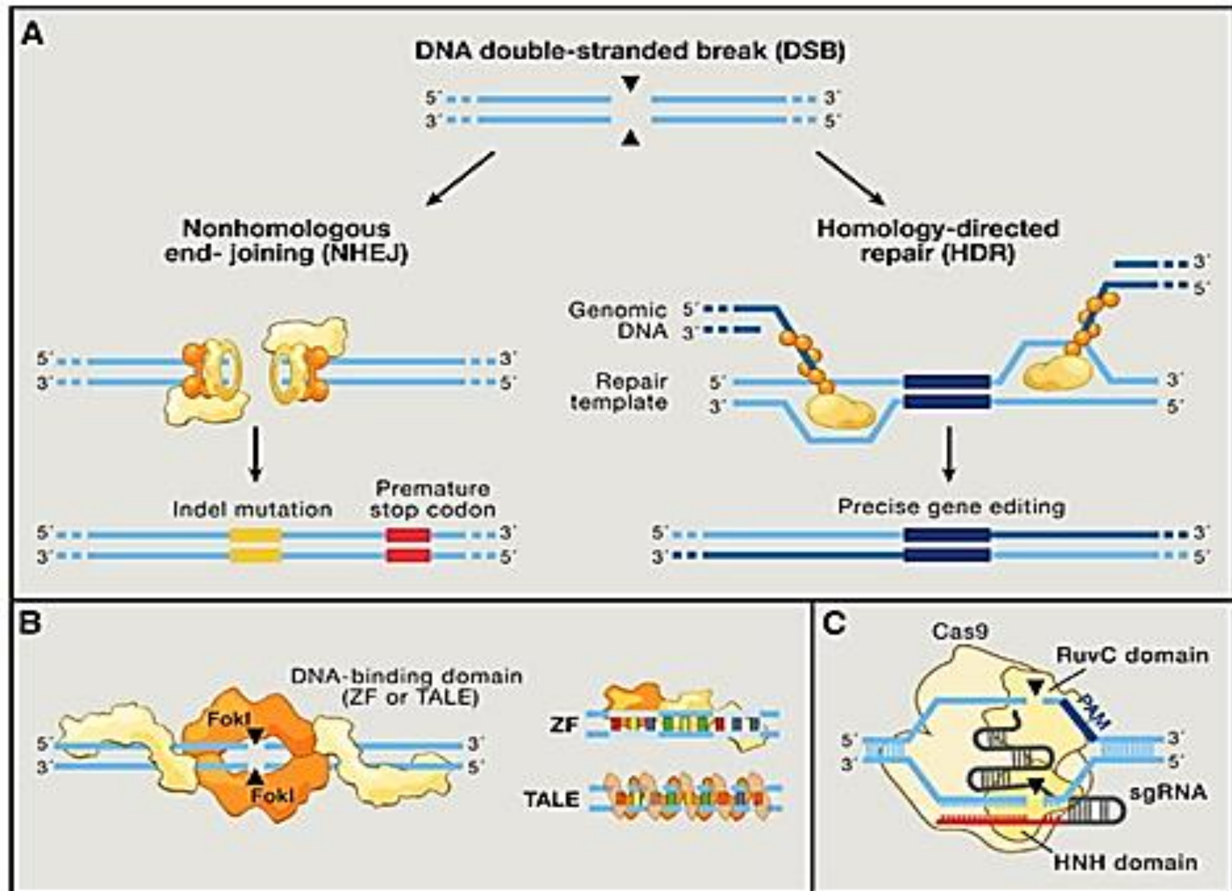

Figure 1.7: Genome editing techno lies exploit endogenous DNA Repair [29].

One of the breakthroughs in genome manipulation was the creation of homologous recombination (HR) gene targeting which incorporates an exogenous repair template containing donor site sequence homolog (Figure 2) [29]. DNA double-stranded breaks (BSB) were usually repaired by Non-homologous end- joining (NHEJ) or homology- directed repair (HDR). Heterodimers bind to (DSB) ends in the error prone (NHEJ) pathway and act as a molecular scaffold for the related repair proteins. Additionally, during the initial step of (HDR), Rad5` proteins that bind DSB ends, recruiting accessory factors that guide genomic recombination with the homology arms on an exogenous repair template. By passing the corresponding brother chromatid makes specific gene modifications easier to implement.

The instructiveness of their plasmid design and construction is an advantage the CRISPR/cas9 system offers over other mutagenic techniques such as ZFN and TALE. The specificity of CRISPR/cas9 for each target site relies on the formation of a sgRNA ribonucleotide structure and the specified DNA and compared to protein on DNA recognition. CRISPR/Cas9 is convent programmable by modifying the sgRNA reference sequence (20 nucleotides in the natives (RNA) of any appropriate DNA strand. In addition, CRISPR can change high fidelity genetic targets while ZFN/TALE is vulnerable to CpG methylation. For the last, the monomeric cas9 protein and any number of different sequence specific gRNAs can easily be achieved with multiplexed genome editing with the CRISPR/cas9 library. The versatility of CRISPR/cas9 programming and its ability to identify multiplexed targets has fuelled the success of this cost-effective and easy to use technology.

Together these results can help create statistical models for reducing off-target action in gene therapy or other applications that require high precision levels. Understanding cas9 binding and cleavage in the sense of accessibility of chromatin and epigenetic conditions would also allow improved statistical assessment of the specificity of the guideline RNA. Such as sgRNA

for example, may be analysed based on the molecular structure of its off-target sites, which may differ by sequence of guides. Degenerate targeting of transcriptionally silent genes for a cell type or tissue of interest will possibly be preferable to off target sites important housekeeping genes in the coding region.

ssDNA oligos for HDR

DSB at specific genomic sites can lead to changes at the DNA break sites via HDR if a homologous donor DNA exists. Customarily, the HDR donor is a plasmid or a double standard DNA containing long homology arms flanking each side of the target site. Recently, single-stranded DNA oligos (ssDNA, sense or antisense) with shorter homology arms (usually 20 to 90 nt long) are used as donors to make minor, specific improvements, including one-base substitution or a few-base insertions [30]. The potential mutation site is usually in the middle of the arms in left and right homology.

1.4 Change on the Agriculture

It is now possible to envision innovative CRISPR technologies for sustainable agriculture. The comparatively low cost and easy for use of CRISPR method promote ground-breaking work in research and enterprises of all sizes, effectively democratizing the production of crop traits. It is now feasible to consider conducting work on niche crops which have traditionally been overlooked [31]. In addition, instead of extending the resistance of already domesticated crops for the climate and disease, plant species that are already well suited to various conditions may be domesticated with high value traits. One of the companies such as Arvegenix for example used CRISPR technology to improve pennycress oil and meal consistency. The goal is to make pennycress both a cover crop that is used for the oil and feed markets in standard growing seasons and a commodity similar to canola. Farmers will get many benefits from increased income if effective while also reaping the benefit of using sustainable crops cover. Many examples are likely to come from using synthetic biology CRISPR technique for selective endogenous gene evolution to produce new beneficial properties, such as the ability to repair atmospheric nitrogen now present only in legumes.

To avoid past mistakes in this sector, the biggest possible pitfall in agricultural use of CRISPR technology is not scientific societal recognition and government oversight. Most planned uses should produce `nature-identical` characteristics that are characteristics which could also be obtained from standard plant breeding. Though it is true that CRISPR technology will be used for various purposes, let's imagine that it will be very restrictive, for example, to add exogenous genes. We do assume that these uses can be easily separated, such that obviously similar CRISPR implementations do not need to be equated with GMOs. Despite this, due to the volatile global regulatory climate, trust in the application of CRISPR tools in agriculture remains minimal. To resolve this will take a political will to follow a consistent stance on CRISPR technology and aim for some sort of consistency between countries.

1.5 Effect of CRISPR on Today Society

Plants and crops are risky towards pests and pathogens, bacteria, fungi and insects which will affect its healthiness and life expectancy. Plants and crops without any protection against these threats are easily infected with disease caused by pests and microorganisms. According to research, the worldwide crop production loss is about an average of 26% annually because of pre-harvest pests and pathogens.

In the olden days, without the presence of genetic resistance in agriculture, the production of foods depends on the chemical control of pathogens. Regardless of the effectiveness of using chemical control towards plants protection it produces a bad side effect to the environment. There is an alternative way to protect the crops and at the same time protect the environment which is by using modern synthetic chemicals. However, it can be used in advanced agricultural production systems and the cost is very high [32].

In the previous years, breeding programs were focused on the natural identification and induced mutant alleles for resistance and its combination into genotypes through breeding techniques. The method of mutation breeding showed positive results where the disease resistance was improved, and this method was used to produce more new crop varieties at the same time. Throughout this method, numerous mutants have been developed through the induction of mutants and plants show its enhanced resistance towards disease.

Genetic engineering techniques are commonly applied in fruit cultivations, as they enhance essential agricultural characteristics

such as the resistance to biotic and abiotic stresses and fruit quality. Recombinant DNA technology allows for the transition of the target genes to fruit cropping from any organism, plant or microorganism, and provides new genotypes and breeding-based phenotypes with improved possibilities for fruit yield. In results, it will boost the consistency of the fruit and increase shelf life. The recombinant DNA technology produced an organism which is called genetically modified organism (GM). The change in its DNA caused its maturation to be slowed down and stopped its softening after picking. Nonetheless, regulatory approval processes mainly affect the production of new GM crops as the main purpose of the approval program is to avoid harm to human health and the environment and avoid financial losses [33]. This may result in very high costs for approving new GM crops and could also slow marketing of goods under regulatory requirements. Therefore, the CRISPR-technology, clustered frequently interspersed, is a safer option.

1.5.1 CRISPR Technology to Society

The CRISPR system is an innovative adaptive immune mechanism for protection against invasion by bacterial and exogenous plasmids, found in bacteria and Archaea [34]. Since the CRISPR-Cas method was designed to successfully alter plant genomes in 2013, several attempts have been made to make it a better tool. Gene editing is commonly used in plant breeding for the development of transgenic plants for implementation of new resistance genes to crop pests and diseases.

1.5..2 Improvement and Changes to Plant and Crops Properties

A Resistance to Biotic Stresses

Biotic stress involves viruses, bacteria, fungi and insects which may attack and infect plants [35]. CRISPR-Cas9 technology has been employed to obtain disease-resistant plants [36]. CRISPR-Cas9 has also been used against viral, fungal and bacterial infections that contribute to significant tomato losses. For fighting virus's infection 2 strategies have been used which are designing sgRNAs and targeting the virus genome directly through sequence complementation and modification of tomato genes conferring antiviral properties. The CRISPRCas9 program was used for growing viable virus resistant plants over many generations. This technology was also used to eliminate main genes involved in systems of resistance, in order to verify if these genes may grant virus immunity.

In the absence of adequate agricultural chemicals, unidentifiable asymptomatic infections can make plant pathogens difficult to manage and the use of genetic resistance against these pathogens the most successful technique [37]. Pseudomonas syringae is the source of the bacterial spot disease, reducing their production and marketability in tomato plants. Since (JAZ2) aids defence, CRISPR-Cas9 researchers have used JAZ2 dominant tomatoes. Banana streak virus is a big problem in bananas growing in tropical and subtropical countries. Therefore, CRISPR-Cas9 focuses on their genomes, one technique for enhancing the resistance to viruses.

B Resistance to Abiotic Stresses

Dryness, floods and heat and cold, especially those in the sense of climate change, present a significant risk for organisms, mostly plants. Conventional breeding strategies have greatly improved crop yield, but new methods are required to further boost crop productivity as food demand is increasing and CRISPRCas9 is the most viable technology [38]. Since tomatoes are a cold-sensitive crop, the quality of its fruit is easily spoiled by cold stress. Li et al. discovered that C-repeat binding factor 1 (CBF1) can prevent plants from cold injury, as the cbf mutant produced by CRISPR-Cas9 showed more extreme signs of freezing and more electrolyte leakage. MAPK3, which participates in resistance against gray mild disease, also contributed to tomato drought response by providing protective cell membranes from oxidative damage [39]. The inactivation of endogenous banana streak virus was carried out using CRISPR-Cas9 system and compared to the un-edited control, 75% of the edited plants remained asymptomatic [39].

Since the first implementation, CRISPR-Cas9 has revolutionized seed breeding the main achievements were the development of fruit crops which resist disease and adapt to the environment besides improving fruit quality. Even so, the rise in CRISPR-edited plants faces socio-political challenges, including public acceptance and government regulation. More countries may wish to promote a constructive and inclusive attitude towards CRISPR-edited crops by more advances in CRISPR technology and developing an evaluation framework.

1.5.3 Ethical Impacts on Society

A Balance of Advantages and Losses

One critical science ethics concern is that advantages must outweigh losses. Risks must be given greater consideration because they can affect human organisms or the environment. The use of the CRISPR / Cas9 technique includes risks as it can produce harmful target mutations. One issue is that large genomes may contain several similar or extremely homologous DNA sequences to the desired target DNA sequence. CRISPR / Cas9 can also distinguish certain unintentional sequences which can result in cell death or transformation by triggering mutations; attempts to reduce off-target mutations have been made however further progress is required especially for accurate changes to therapeutic interventions. The effective safe supply of CRISPR-Cas9 to cell types or tissues that are difficult to transfect and invade is another significant challenge.

B Ecological equilibrium

When conducting experiments using CRISPR / Cas9-based RNA-guided genetic drives, details must be studied keeping the target results into account. Given the genetic drive still operates in produced organisms, mutations from goals are likely to occur and each generation will be increased. If there is a chance that genes will be passed to other animals, there is a danger that changed genomes will be transmitted to similar bodies through political borders. It may be hard to monitor the dispersion of the gene drive characteristic. In fact, the extinction of the entire population controlled by gene drive may have drastic impacts on the balance of the ecosystem. For example, other plague can grow. Scientists warned of the dangers of unintentional release of genetically engineered laboratory species in the environment [39]. Safety precaution is a must to prevent the spread of organisms that can cause ecological damage and affect human health.

C Regulation for Consumer Regarding CRISPR/Cas9

The CRISPR / Cas9 technique's success in producing specific genetic modifications makes it more difficult to classify a genetically modified organism and control it on the market. Regulatory agencies like the US Food and Drug Administration should authorize for customers every genetically modified organism, but it is not simple how to cope with an expanding CRISPR / Cas9 in industry possibly. The regulation of patenting is another matter. A lot of business interests are involved, transgenic species have been patented for agricultural use. Besides, human gene sequences for medicinal use have also been patented; this has contributed to tremendous advancement of biotechnology. However, lawsuits can emerge from the practice of patenting. Biotechnological firms have also encountered confusion and conflict over the patenting of CRISPR / Cas9 for medicinal use in humans.

1.5.4 Genome editing for enhancement

The potential for non-therapeutic treatments using gene editing is another legal concern to be addressed. For health purposes, the use in germ lines is banned. Nevertheless, the flexibility of the CRISPR / Cas9 therapies helps somatic cells to interact and fit evolution with our interest in life. Most phenotypic characteristics have a genetic aspect that can be influenced in addition to the environment. This technique can be used to enhance athlete performance or discourage or reduce the violence of athletes. Generally speaking, gene therapy seeks to enhance the health of an individual, although it may happen where the criminal justice system will require gene editing for serial offenders or dangerous unsafe perpetrators due to the abuse. Socially, there would be a question if people or persons, for example in their intellectual capacity, will be improved physically relative to others.

Many have advocated for a national debate, but other important issues must still be addressed with respect to this method on the financial, ethical and legal implications of genome-editing technologies in human germline. In addition to humans, plants, animals and environment, the use of CRISPR / cas9 renews many other societal and ethical concerns such as taking into account the anti-malignancy concept in risk assessment, health issues to prevent biological damage, or the application of this technique to develop genetics.

CONCLUSION

The novel genotypes are no longer susceptible to the resistance gene because of the large risks of mutation in plant pathogens. CRISPR / Cas9 of *Streptococcus pyogenes* (SpCas9) has quickly

acquired a significant position in numerous applications for plant science and in several other fields. A single-guide RNA (sgRNA) in the CRISPR / Cas9 network can bind to Cas9 and link it to different DNA sequences. The simultaneous modulation of several genes with a common molecular structure is one of the key advantages of CRISPR / Cas9 technologies with respect to MN, ZFN or TALEN.

It is now possible to envision innovative CRISPR technologies for sustainable agriculture. The comparatively low cost and simple to use CRISPR process fosters innovative work in science and companies of all sizes, essentially democratizing crop development. Consideration of doing work on small crops that have historically been ignored is now feasible. For several years the CRISPR / Cas9 software has been used to cultivate productive plants immune to viruses. This research has also been used to delete large genes implicated in resistance mechanisms, to test whether certain genes may offer protection to viruses.

Using the CRISPR / Cas9 method entails dangers since it may create dangerous mutations in targets. One concern is that broad genomes which contain many sequences of DNA that are identical or highly homologous to the same target DNA sequence. CRISPR / Cas9 may also discern such unwanted sequences that can result in cell death or transformation through causing mutation attempts to minimize off-target mutations have been produced, but more improvement is needed particularly for effective therapeutic intervention improvements.

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