Enhancing Crop Resilience through CRISPR/Cas9-Mediated Development of Disease-Resistant Cultivars

Abstract

A major issue in agriculture is the protection of crops against diseases and pests. Plant breeding has been primarily responsible for the growth of disease-resistant cultivars. The use of gene editing techniques in plant breeding is essential for obtaining desired features. Clustered Regular Interspaced Palindromic Repeats (CRISPER)/Cas9 (CRISPR-related protein) is a new advancement in gene editing technology. It can be utilised in plant defence mechanisms against pathogen attack by recognising the bacterial immune system and destroying invasive pathogen genes. Advances in plant breeding through CRISPR/Cas9 integration have helped develop cultivars including hereditary resistance to bacterial and viral diseases. Future crop generations can acquire CRISPR/Cas9-mediated transgene resistance if the Cas9/sgRNA transgene has been isolated in the F1 generation. Cas9/sgRNA transgene separation makes CRISPR/Cas9 safe for use in plant breeding. Although CRISPR/Cas9 has proven to be a wonderful tool to revolutionize plant breeding and develop various disease resistant varieties, its effect on many plant physiological processes remains to be thoroughly investigated.

Keywords:CRISPR/Cas9, Gene editing, Genome,Plantbreeding,Resistancebreeding.

Introduction

A major challenge is to protect crop varieties against current pests and diseases and to improve crop varieties to increase yields. shortage of disease-resistant crop varieties is the main point why farmers suffer from reduced agricultural production. To generate disease-resistant crops and assure food security, breeding for disease, insect resistance, and higher yield is helpful [31]. Resistance breeding, which uses a variety of cutting-edge molecular methods including genome editing in transgenic plants, aims to improve crops by increasing their resistance to pests and illnesses. Breeders can cross species to add genes from unrelated plants and other creatures into crops thanks to transgenic technologies [31]. In order to fulfil their nutritional demands, the expanding population—which is expected to number 9.8 billion by 2050 as a result of population increase worldwide [4]—must produce an excessive amount of food. Plant pathogens, which include bacteria, viruses, fungi and parasites, threaten global food security [2,30]. To increase crop yields and meet world food demand, it is important to increase plant resistance [11]. The constant struggle between plants and diseases to protect each other is well known [16,42]. To defend against infection, plants have evolved "template-triggered immunity (PTI)" and "effector-triggered immunity (ETI)" [17]. PTI is usually rapidly activated by “pathogen-associated molecular patterns (PAMPs)” via “pattern recognition receptors (PRRs)” [32,25].

Resistance breeding heavily relies on genetic diversity. A essential component of using the idea of resistance breeding is developing resistance and adding genetic variety to harmful genes [43]. These findings have led to the employment of various gene editing methods to create genetic variations. The CRISPR (Clustered Regular Interspaced Palindromic Repeats)/Cas9 (CRISPR-associated protein) bacterial immune system, an RNA-guided technology for effective gene control and editing, is one recent development [39]. Other gene editing methods exist, such as transcription activator-like effector nucleases (TALENS) and zinc finger nucleases (ZFN), although they are less effective than CRISPR/Cas9 owing to their size and requirement for protein pairs. Double Strand Break (DSB) is

caused by parallel strands [7]. Due to its advantageous characteristics, such as exact specificity, multiple gene editing, little off-target effect, improved efficiency, and simplicity, CRISPR/Cas9 is seen to be a very promising technology for crop gene editing [29]. This special bacterial system collects foreign or invasive DNA fragments and employs them to identify and further break down invasive DNA or RNA sequences. By locating and destroying invader pathogen genes, CRISPR/Cas9 technology can be utilised to benefit plant defence mechanisms against disease assaults.

It is possible to create strains that are resistant to illness using CRISPR/Cas9. With its capacity to act as a sequence-specific nuclease, CRISPR/Cas9 represents ground-breaking work in the study of resistance replication.The application of CRISPR/Cas9 technology in plant breeding to create disease-resistant cultivars is covered in this review. Using the keywords "CRISPR/Cas9", "resistance breeding", and "plant breeding," studies were discovered in the online literature databases scops.com and scholar.google.com for this review. The use of the CRISPR/Cas9 system for gene editing and gene modification is covered in the body's first section. The use of CRISPR/Cas9 for disease resistance is covered in the next section. The usefulness of CRISPR/Cas9 in plant breeding is examined in the third section from the standpoint of disease resistance. This study provides details on the development of CRISPR/Cas9 and its application in resistance breeding in an effort to aid researchers in their future work. In the CRISPR/Cas9 system, the guide RNA binds to the DNA, and pre-designed sequences in the RNA direct the Cas9 enzyme to cut the DNA strands in the right places. DNA cutting is done by removing and adding the necessary sequences to the target DNA.DNA recombination causes mutations and fixes DNA breaks. But occasionally it also results in undesirable alterations that further bind to the target DNA. This may cause guiding RNA to attach to the incorrect places and break non-target DNA strands, which may result in erroneous mutations and the loss of certain significant DNA sequences holding crucial information. However, because the Cas9 enzyme can be controlled to target various sites on DNA by altering guide RNA sequences, researchers are attempting to employ CRIPSR/Cas9 more accurately [45].

The most significant and essential danger to agricultural progress and global food security is plant diseases. Numerous diseases are responsible for 20–50% of crop losses worldwide, according to FAO estimates released in 2015 [14, 20]. In addition, due to their widespread and irregular use, plant pathogens also increase resistance to various pesticides and other chemicals [1]. Therefore, it is imperative to prevent their usage in food production in order to minimise the harmful environmental impacts of pesticides and to safeguard animals and flora. On the other hand, a successful and sustainable approach to sustainable agriculture is the development of disease-resistant crop types through plant breeding. In recent decades, traditional breeding of disease-resistant cultivars has been effective, but it has a number of drawbacks, including labour intensity and resistance (R) gene introgression. Additionally, lack of genetic diversity in the plant population, crossover or independence between the two most suited plants, and transfer of unwanted genes or characteristics with desired resistance genes or traits are the key drawbacks of conventional resistance breeding [15]. Therefore, keeping up with the development of pathogens and the rising demand for food is

extremely difficult for conventional processing, particularly in the age of global climate change.

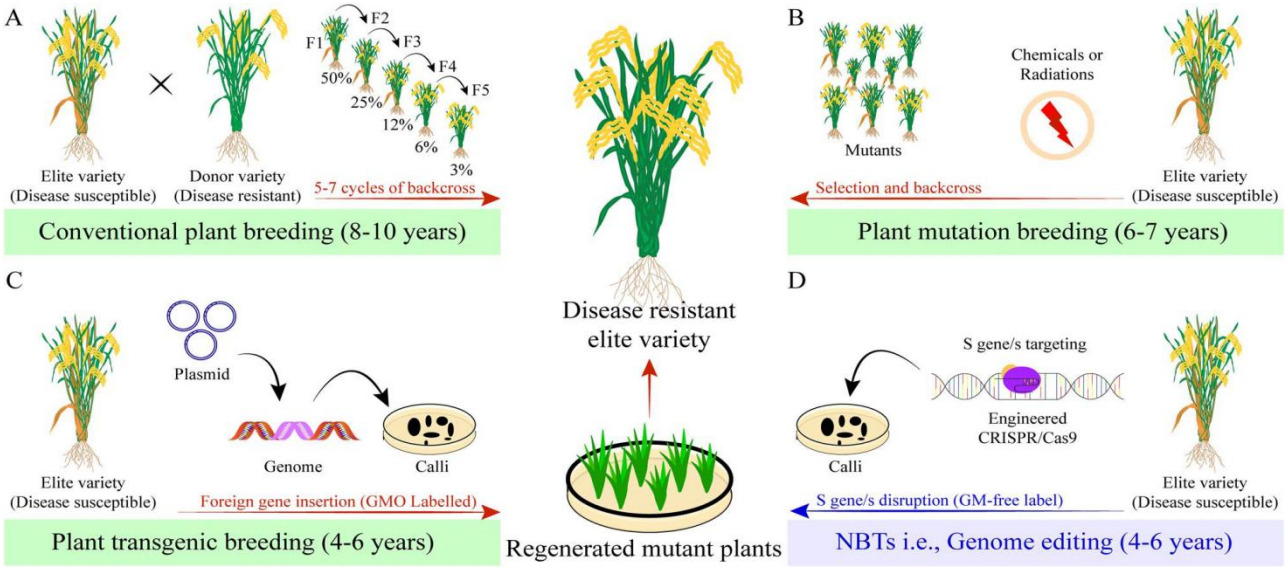

Figure-1: An illustration of a comparison Chen et al. [8] made between plant breeding and mutagenesis techniques for agricultural development (using rice as an example). {(A) A traditional method of plant breeding known as crossing has long been used to improve plant traits, such as disease resistance, by crossing a donor parent line with a recipient parent line that has the desired trait. After many repeated backcrosses and severe cycles of selection, an exceptional progeny with the desired trait or characteristics is selected. This method has many disadvantages, including being difficult, time-consuming and less effective. (B) Mutagenesis refers to chemical or physical mutagenesis. Often, various mutagens are applied to the seeds to cause mutations in the genome of the plant. Mutants generated by this method are subjected to strict selection to assess the desired phenotype. This is another method of finding and mapping new genes in the genome. In addition, the process is long and it takes about 6-7 years to achieve the desired results. Random changes in the genome, which can sometimes be difficult to detect and predict, are one of the most important disadvantages and limitations of this technique. (C) Transgenic breeding, which involves the transfer of a desired gene from one genome to another, has been successfully used to improve various agricultural traits (eg, Bt cotton, Bt corn, etc.). The targeted trait or traits are enhanced when the gene of interest is accurately and precisely inserted into the genome of the host elite culture. However, because someone's genome has been altered by adding foreign DNA or elements, these plants are genetically modified. One of the biggest challenges with GMOs, among many other problems, is their lack of acceptance by the public and many botanists around the world. (D) A sustainable future for plant development requires the use of new methods of genetic modification, often referred to. as "new". (breeding) techniques "such as genome editing are both promise and hype. Plants are improved by targeting and knocking out specific negative regulators or genes in a specific gRNA-based target and by rearranging chromosomes in the genome of elite types. Compared to other methods ., improving traits with genome editing techniques such as the CRISPR/Cas9 system, a reliable, efficient, time- and labor-saving and cost-effective method. Furthermore, because the plants produced using this approach don't include foreign DNA, they may be labelled as non-GMO and escape GMO regulations.}

Even while mutant breeding and transgenic technology are in use, they have several drawbacks that make them less demanding today (Figure 1). These issues with our existing agricultural methods point to the necessity of introducing better and more efficient ways (such as genome editing technologies, or GETs) of producing resources for breeding novel crops. Clustered regularly interspaced short palindromic repeat (CRISPR) technology is one of the latest GETs that is frequently used to create ideal plant material for long-term food production [51].

Tool for Gene Editing and Gene Manipulation in Plant Breeding: CRISPR/Cas9

Gene editing and gene modification have long been fascinating study areas for plant breeding and molecular biology. In order to achieve desired features and qualities in an organism, it is vital to regulate and control the expression of certain genes. This process is known as gene regulation, and it is heavily utilised in cultivar development for disease resistance and increased yield. Gene editing is widely utilised in plant breeding to create transgenic plants that include novel resistance genes for crop pests and illnesses. When transgenic plants are created using gene editing technology for plant breeding, all of the genetic variety that is now accessible is used without any restrictions from natural cross barriers, resulting in plants that cannot be produced using traditional breeding techniques. For molecular scientists and plant breeders, various methods for gene editing and gene modification are crucial tools for integrating the crucial genes in the genomes of significant crops [29]. The binding domain and the impact or domain make up the basis of gene editing. The effector domain aids in the cleavage of DNA at the target site and controls transcription, whilst the binding domain aids in the

recognition and binding of sequence-specific DNA [45].

As an adaptive immune system that many bacteria possess, the CRISPR/Cas9 system functions in a succession of phases as seen in Figure 2. Initially, CRISPR-containing organisms recognise foreign nucleic acid and pick up minute pieces of DNA from bacteriophages and plasmids that have invaded their territory. The host then inserts the obtained bits as pauses between brief DNA repetitions into its CRISPR locus. Protospacer Adjacent Motifs (PAMS), a brief segment of conserved nucleotides, serve as recognition motifs for the incorporation of DNA fragments into the spacer [29]. After the Cas proteins are expressed, the spacers obtained by CRISPR are translated into pre-CRISPR RNAs (pre-crRNAs), which are subsequently cleaved and matured to create CRISPR RNAs (crRNAs).

These crRNAs have a spacer sequence from a prior foreign nucleic acid that aids in the identification and cleavage of the invading genome. This helps to safeguard the host cells by matching the invading genome with the spacer sequence. In spite of their practical application, mutation breeding and transgenic technologies have several drawbacks that make them less popular nowadays (Figure 3). These issues with our existing agricultural methods highlight the requirement for newer, quicker technologies (such as genome editing techniques, or GETs), to provide resources for breeding new types. Clustered regularly interspersed short palindromic repeat (CRISPR) is a well-liked technique being applied to create acceptable plant materials for long-term food supply among the rising GETs [51].

Gene editing and gene modification tools for plant breeding using CRISPR/Cas9

In plant breeding and molecular biology, gene editing and modification have long been fascinating issues. Gene regulation is a crucial component of regulating and managing the expression of particular genes to generate desired features and characteristics in an organism. It is frequently utilised in breeding disease-resistant and more productive types. It is common practise in plant breeding to utilise gene editing to create transgenic plants and add new resistance genes against crop pests and illnesses. Genetic engineering, which creates transgenic plants by using all genetic diversity for plant breeding without being constrained by natural crossing barriers, produces plants that are not possible to breed using traditional breeding techniques.Molecular scientists and plant breeders can use a variety of gene editing and gene modification techniques to incorporate crucial genes into the genomes of crucial crops [29]. The binding domain and the effector domain make up the basis of gene editing. The effector domain enables DNA cleavage at the target region and controls transcription, whereas the binding domain assists in sequence-specific DNA recognition and binding [45].

The sequential nature of the CRISPR/Cas9 system's operation in many bacteria's adaptive immune system is seen in Figure 2. CRISPR-equipped organisms initially detect foreign nucleic acids and take tiny DNA pieces from bacteriophages and plasmids that have invaded their territory. The acquired fragments are subsequently inserted by the host between short DNA repeats in its CRISPR locus. Protospacer Adjacent Motifs (PAMS), a brief set of conserved nucleotides, act as recognition motifs to draw DNA fragments to the spacer [29].The CRISPR-acquired spacers are subsequently translated by Cas proteins to create pre-CRISPR RNAs (pre-crRNAs), which are then cleaved and matured to produce CRISPR RNAs (crRNA-s). These crRNAs have a spacer sequence from an earlier foreign nucleic acid that aids in identifying and cleaving the invading genome, which helps safeguard host cells [45].

Disease Resistance and CRISPR/Cas9 Use

Through guide RNA (sgRNA) technology, the CRISPR/Cas9 system may be utilised to breed disease resistance in plants. Trans-encoded CRISPR RNA (tracrRNA) and crRNA combine to create sgRNA

[35]. The RNA-guided nuclease that Cas9 and sgRNA create controls sequence-specific cleavage and editing in the target genome [23]. The pre-designed sequences in guide RNA, which contains around 20 base pairs and is complementary to target DNA and aids in the binding of guide RNA to strands of target DNA, define the site-specific cleavage activity of the sgRNA-Cas9 complex [29,45]. (Figure 3).

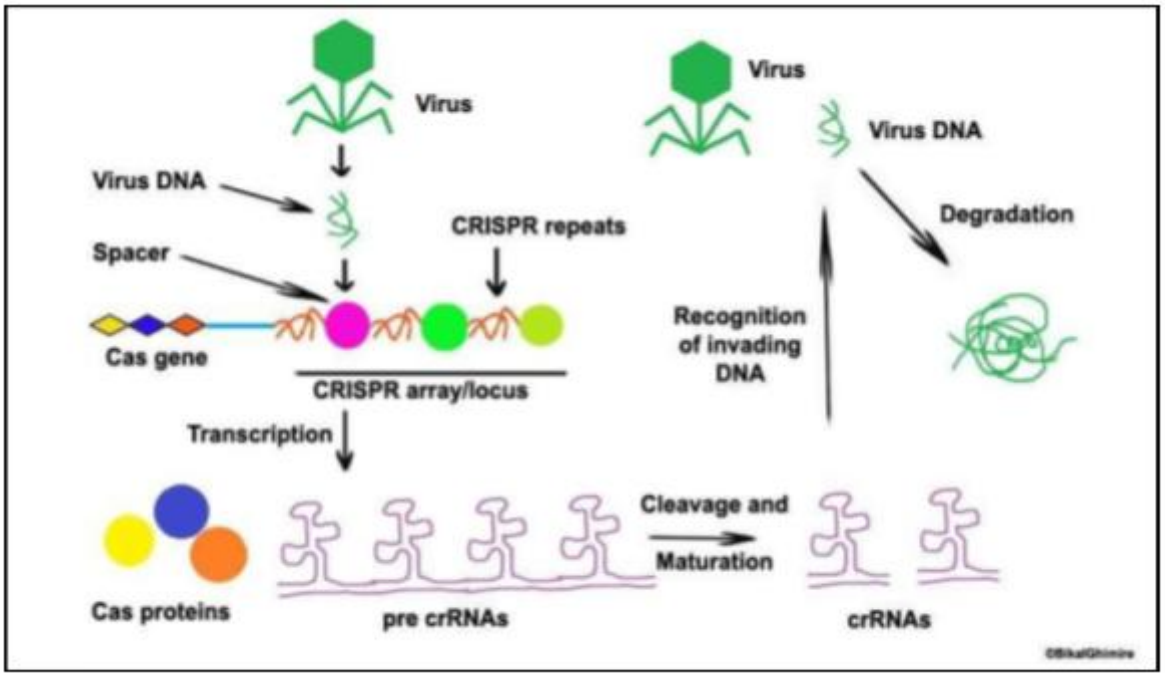

Figure2: The CRISPR/Cas9 system's operation [29]. An organism that has the CRISPR gene recognises the invasive DNA, removes a segment, and inserts it between the CRISPR repeats. Upon maturity, crRNAs are produced through cas gene transcription. These crRNAs can quickly distinguish the foreign DNA and break it for destruction, defending the host. The CRISPR/Cas9 system has been studied in different articles and reviews, on which the picture is based.

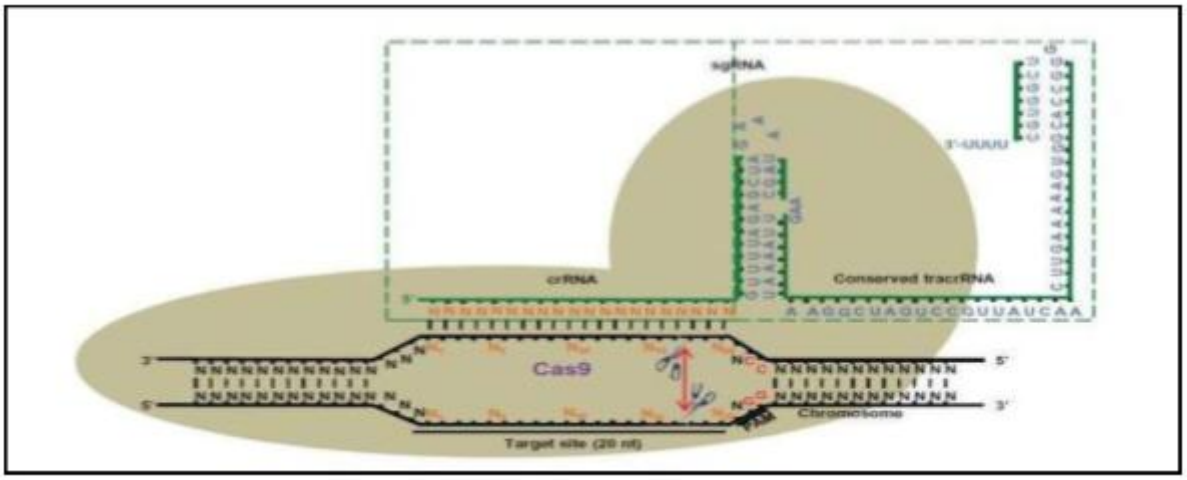

Figure- 3: The backbones of genomic DNA and RNA molecules are shown by the green and black lines, respectively, in this schematic representation of the CRISPR/Cas-mediated cleavage process. Crack areas are shown by the red arrow. The terms Cas9, (CRISPR)-related protein 9, crRNA, PAM, single guide RNA, and tracrRNA all refer to the CRISPR system. This number is from [29].

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1. Specific bacterial DNA sequences may be altered using sgRNA as a binding domain, and RNA-guided nucleases can break invasive foreign DNA like phages in a way that is sequence-specific [9]. The guide RNA attaches to the DNA, and specially constructed sequences in the RNA tell the Cas9 enzyme where to cut the DNA strands. DNA cutting involves taking out and inserting necessary sequences to the target DNA [45]. By delivering sgRNA and Cas9 to target cells for gene

transformation, this technology can be investigated to develop resistance to certain diseases in plants. Several techniques, including electroporation, plasmid-mediated transformation, agrobacterium-mediated transformation, shotgun approaches, particle bombardment, and polyethylene glycol-mediated transformation, are utilised to deliver sgRNA and Cas9 into plant cells [22]. However, gene transformation by Agrobacterium is straightforward and often employed in numerous investigations. The use of CRISPR/Cas9 technology to shield plants from Gemini viruses was described by Baltes et al. [5]. To test the effectiveness of CRISPR/Cas9 against the tomato yellow leaf curl virus (TYLCV) in Nicotiana benthamiana plants, Ali et al. (2015) [3] conducted experiments. Their findings revealed strong evidence of antiviral DNA interference using tobacco driver RNAs engineered by Agrobacterium tumefaciens.A rattlesnake virus (TRV) containing a TYLCV-specific sgRNA invaded Agrobacterium tumefaciens and invaded that plant. TYLCV accumulation was lower in sgRNA-infiltrated plants than in control plants [3]. Similar outcomes were attained when beetroot strong curl virus was introduced to Nicotiana benthamiana and Arabidopsis thaliana using CRISPR/Cas9 technology along with Agrobacterium-mediated gene transformation [21]. By blocking the viral gene from reaching the replication protein, cutting viral double-stranded (ds) DNA to inhibit replication, or creating error-prone mutations in the viral genome, Cas9/sgRNA disruption hinders viral gene replication. [3]. Additionally, additional viral DNA with identical sequences is targeted by the sgRNA. Three viruses, TYLCV, beetroot loop top virus (BCTV), and Merremia mosaic virus (MeMV), which correspond to an invariant viral sequence in the CRISPR intergenic region, were successfully targeted by Ali et al. [3]. Variable crRNAs can be utilised to create sgRNAs with a variety of targets since the crRNA sequence controls the cleavage of particular DNA targets [29]. Table 1 lists the plant species whose gene transformation has been integrated with CRISPR/Cas9, which induces plant species resistance to various diseases.

Traditional breeding has made tremendous strides towards enhancing cotton's ability to respond to the environment at hand for improved quality and production. Due of the lengthy breeding process, random gene insertion, and frequently deceptive phenotype-based selections, conventional breeding techniques are handicapped. The advent of recently created CRISPR/Cas9 genome editing technology heralds a new era in the pursuit of accurate cotton breeding. A naturally occurring genome editing tool called CRISPR/Cas9 was adapted from the prokaryotic adaptive immunological defence system. CRISPR/Cas9 has been quickly adopted for application in a variety of domains, including gene function research and precise breeding, due to its simplicity and durability. CRISPR/Cas9 has been immediately embraced by numerous cotton research institutions and biotechnology enterprises worldwide since it was first used in cotton in 2017, when Li and colleagues modified an endogenous functional gene MYB-15 similar to cotton. Currently, CRISPR/Cas9 is being extensively employed to explore gene function and cotton improvement, including fibre development, resistance to abiotic and biotic stress, cotton morphological alteration, and secondary metabolism. CRISPR/Cas9-based precise breeding will greatly improve cotton breeding and productivity during the following ten years[52].

Efficacy of CRISPR/Cas9 in reproductive resistance

By deleting the target sequence and adding the desired gene, this approach causes a mutation in the target DNA. It aids in the mutagenesis of inaccessible genes, the editing of several genes, and the generation of huge gene deletions, all of which serve to advance plant reproduction [28]. The Cas9/sgRNA transgene may be deleted to make the plant transgene-free, and this exact mutation of the plant genome can be passed down generations after generations as illustrated in Figure 4 [49].

Table-1: List of Plant Species Resistant to Diseases Caused by CRISPR/Cas9 Integrated Gene Transformation (Based on Previous Research Papers)

PlantSpecies	Mediumfor gene transformation	CRISPR/Cas9integratedwithgene transformationinducedresistanceagainst	References
Nicotianabenthamiana	*Agrobacteriumtumefaciens*	TYLCV (TomatoYellowLeafCurlVirus),BCTV (BeetCurlyTop Virus),MEMV (Merremia MosaicVirus)	[3]
Nicotianabenthamiana	*Agrobacteriumtumefaciens*	BeYDV (BeanYellowDwarfVirus)	[5]
Nicotianabenthamiana, Arabidopsisthaliana	*Agrobacteriumtumefaciens*	BSCTV (BeetSevereCurlyTop Virus)	[21]
Nicotianabenthamiana	*Agrobacteriumtumefaciens*	CaLCuV (CabbageLeafCurlVirus)	[50]
Oryzasativa	*Agrobacteriumtumefaciens*	BacterialBlight	[22]
Triticumaestivum	*Particlebombardment*	PowderyMildew	[46]
Oryzasativa	*Gibson Assemblyand Protoplasttransformation*	Ricedisease	[48]

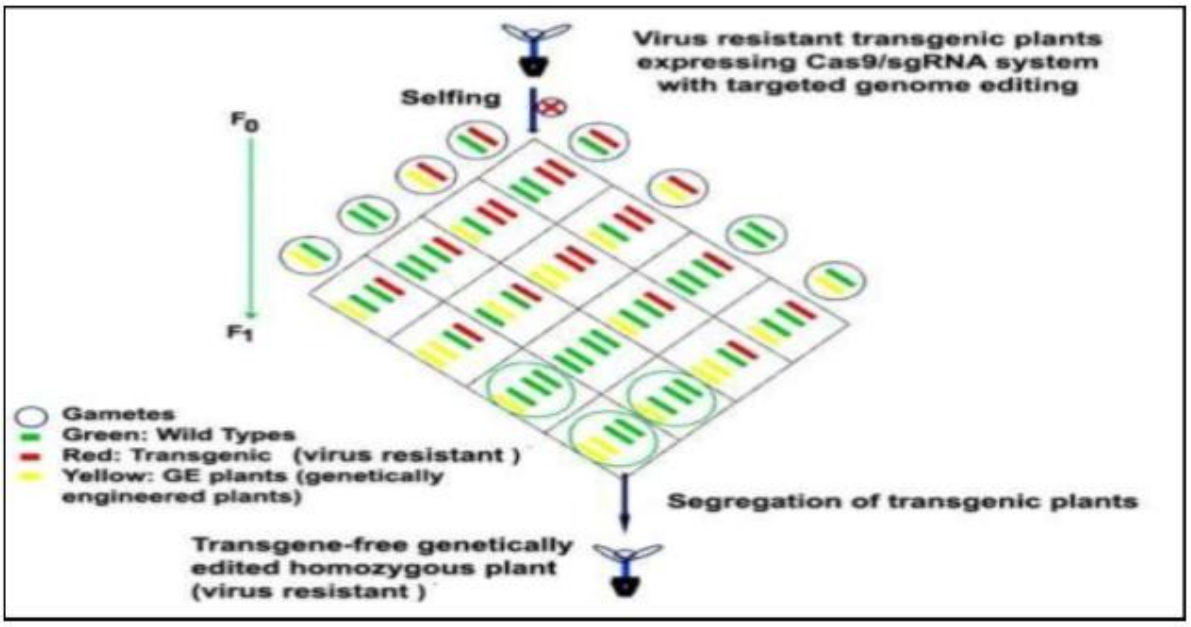

Figure- 4:A series of crops that have been genetically modified (GE) to withstand viruses but lack transgenes. {Transgene-free virus-resistant homozygous mutants with the desired modification at the target gene locus can be selected from the F0 generation plants themselves. Isolation of the transgene in the next F1 generation avoids possible modification of the genome by CRISPR/Cas9 [27]}.

With the help of this technique, a transgenic variant conferring resistance to a particular viral, bacterial, or pest disease can be integrated into the plant genome, allowing the generation of subsequent generations of plants resistant to specific viruses or diseases [46]. Xu et al. (2015) [49] found the development of transgenic rice with desirable traits.In the F1 generation, distinct offspring are produced by selfing plants with wild-type genes, virus-resistant transgenes, and genetically modified (GE) plants. Future generations won't undergo undesirable CRISPR/Cas9 editing thanks to the F1 transgene's isolation. The selection of homozygous transgene-free plants with resistance genes in the F1 generation is made in order to continue breeding and develop cultivars resistant to particular viral, bacterial, and insect

diseases. [26]. 3. Self-fertilization of F1 plants in autogamous plants can result in 25% of F2 plants without the transgene [28]. However, in self-compatible or dioecious perennial woody plants, controlled crosses between male and female primary transformants with biallelic mutations can produce transgene-free biallelic mutants in 25% of the progeny [6, 28].

CRISPR/Cas9-mediated reproductive limitations

CRISPR-based technology has revolutionized plant biology, and many creative solutions have been developed to solve various problems [41,34]. Increasing plant disease resistance is one of the most significant applications of CRISPR technology [24,10]. Plant pathogen resistance has recently improved [19,40], but there are still a number of issues that must be resolved before CRISPR technology can completely realise its potential in this area. The specificity of the CRISPR-based technologies used today makes, resistance to plant pathogens is limited [38]. Because the target DNA must contain a protospacer flanking motif (PAM) for CRISPR to be specific, creating CRISPR-rooted tools to target distinct pathogens is challenging [33]. Off-target effects can also lead to unwanted consequences, namelybreak down of key genes, breach of non-target regions either initiation of epigenetic changes [37]. This can subvene the whole well-being & growth rate of the plant and weaken the ascendancy of CRISPR-based technology [39]. Another limitation is the delivery of CRISPR-based plant-pathogen resistance mechanisms to target cells [12]. One of the main obstacles is the lack of efficient delivery systems for CRISPR-based tools for cell targeting [36]. This can be a significant barrier, specifically in large applications where delivery costs are prime concern. The efficiency of CRISPR-based techniques is also limited by the appearance of the target DNA [24,7]. When the target DNA is large, it is more difficult to develop a CRISPR tool with efficient pick out ability and the desired result [47]. The number of targets that can be successfully accessed may be limited, which would reduce the utility of CRISPR-based technologies [13]. Another disadvantage of CRISPR-based tools is their limited durability. Unlike conventional breeding methods, the CRISPR-based tool is not permanent and can be reused from time to time to insist its effectiveness. Recycling can be expensive, particularly in large-scale applications [18,42].

Conclusion

Growing community, insufficient land resources, climate change and the emergence of recent pests and diseases have posed serious threats to crop production and world food pressure, challenging food crises. Sustainable agriculture, which uses genetic engineering methods to breed disease-resistant varieties, appears to be a key solution to these issues. CRISPR/Cas9 as a bacterial adaptive immune system can be used as a powerful tool in plant breeding to edit genes to develop desired traits in plants. Integrating this technology in plant breeding facilitates the production of varieties with hereditary resistance to bacterial& viral diseases. The probability to isolate Cas9/sgRNA after the evolution of disease-resistant plants create the use of CRISPR/Cas9 secure in plant breeding. However, different studies on the efficacy of CRISPR/Cas9 on plant physiology have not yet been conducted. CRISPR/Cas9 can cause mutations in viruses and other pathogens that may encounter selection pressure to develop resistance to the CRISPR/Cas9 system. As a result, pathogens can develop invariant

sequences in their genomes that correspond to intergenic sequences in the CRISPR array and develop resistance to CRISPR/Cas9 technology. Also, CRISPR/Cas9 unification can influence a plant-induced defense mechanism to produce volatiles during pest attack. Additionally, there are no research on CRISPR/Cas9's impact on the rate of photosynthesis. In conclusion, the CRISPR/Cas9 system is a useful tool in plant breeding for the creation of numerous disease-resistant types, but its impact on plant physiology and potential viral mutations that can result in CRISPR/Cas9 resistance. The system needs more research.

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