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CRISPR/Cas9 system a novel tool for the management of plant diseases: A Review

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Abstract
The focused on genome altering that objectives genomic successions in a site explicit way has as of late developed as a best biotechnological weapon for boosting resistance in plants against broad phytopathogens. Among different quality altering methods, quality focusing on eminently by CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) has richly stirred considerable energy among farming researchers since its disclosure in 2013. It is a reasonable, simple, efficent and quickly flourshing procedure that happens in nature as a prokaryotic safe framework and presents protection from remote attacking hereditary components, for example, plasmids and bacterial infections by intruppting good have pathogen association. The most significant preferred position of this strategy over other quality altering strategies is that it empowers exact genomic adjustments effortlessly and increasingly successful way, diminishing off target impacts and can likewise be fit for altering various genome site all the while. The CRISPR/Cas9 procedure offers the chances to revise the effector-target succession for keeping away from effector-target sub-atomic correspondence and furthermore to alter effector-target advertisers for expanding the statement of objective qualities and consequently occupied with the obstruction procedure. Other than it's across the board job in infection and bacterial malady opposition, as of late its possibility in contagious sickness the executives has additionally been accounted for. The method showed plant obstruction massively by following up on the resistance parts and demonstrated as a brilliant and fundamental methodology for maintainable agribusiness. The CRISPR/Cas9 is quickly advancing strategy and its application is continually extending step by step. In this audit, we have outlined the CRISPR/Cas9 framework and its role in creating plant resistance.

Keywords: CRISPR-Cas9, genome modifications, disease resistance, sustainable agriculture

Introduction
During the most recent couple of decades, the CRISPR-Cas based frameworks have opened the new time of molecular biology and become the most encouraging instrument among farming researchers for altering and changing genome in across the board creatures particularly plants (Belhaj *et al*. 2015) [1]. The arrival of the CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR Associated protein 9) genome-editing technique enabled researchers to modify genomic sequences in a more precise way (Knott and Doudna, 2018) [8]. By and large, the CRISPR/Cas9 innovation is advanced from prokaryotic organism (a sort II bacterial resistant framework) that follows up on a versatile invulnerable framework and in this way shields these living beings from attacking DNA infections and additionally plasmids. Besides, it speaks to another variety of focused genome altering innovation comprehensively which can be connected broadly to about all life forms in a practical way, including the potential for decreased pesticide use. In CRISPR/Cas9 system, site-explicit change is accomplished by a solitary guide RNA (more often than not around 20 nucleotides) that is corresponding to an objective quality or locus and is moored by a protospacer nearby theme. Cas9 nuclease at that point severs the focused on DNA to create twofold strand breaks (DSBs), which are in this way fixed by non-homologous end joining (NHEJ) or homology-coordinated fix (HDR) instruments. At the point when joined with twofold or multiplex guide RNA plan, NHEJ may likewise present focused on chromosome cancellations, while HDR can be built for objective quality rectification, quality substitution, and quality thump in (Song *et al*. 2016) [15]. The application of CRISPR/Cas9 editing has become a powerful tool for future enhancement of agronomic traits in crops (Mohanta *et al.*, 2017) [11].
Remarkably, CRISPR/Cas DNA editing system is able to achieve efficient gene editing in plants through either transient experiment or transgenic plants. In many cases, Cas9 and gRNAs are introduced inside plant cells by *Agrobacterium*-mediated T-DNA transformation or physical means, such as PEG-mediated transformation of protoplast or biolistic transformation of callus. In CRISPR technology, the Cas proteins, such as Cas9, are RNA- directed endonucleases which are able to recognize and cleave nucleic acids on the basis of sequence complementarities (Brouns *et al*. 2008) [2]. Cas9 can be targeted to specific DNA genomic sequences by engineering separately an encoded small guide RNA (sgRNA) with which it forms a complex. Thus, only a short RNA sequence must therefore be synthesized to confer recognition of a new target.

Method of CRISPR/Cas9 based genome altering
CRISPR-Cas9 is another quality altering innovation that offers the potential for considerable improvement over other

quality altering advances which offers the most dependable and flexible stage to design plant genome in a seguence explicit way. Regardless of its enormous applications in animal sciences, it has now turned into the strategy for decision for genome designing in plants for multipurpose (Puchta and Fauser, 2013) [12]. The CRISPR/Cas technology is initially regulated by the bacterial type-II CRISPR/Cas adaptive immune system that is displayed by the hosts to cleave invading phage or plasmid DNA (Doudna and Charpentier, 2014) [16]. In general, CRISPR-Cas9 framework just requires three segments, i.e., a protospacer-containing CRISPR RNA (crRNA), a trans-actuating crRNA (tracrRNA) and a Cas9 endonuclease for capacity. The Cas9 nuclease is directed by the crRNA tracrRNA duplex to separate any trespassing DNA conveying the equivalent protospacer grouping which is accompanied by a protospacer adjoining theme (PAM). The PAM is completely vital for objective acknowledgment as it conveys the foundation of the crRNA tracrRNA-Cas9 unpredictable and consequitive and progressive base blending between the crRNA and the protospacer (Sternberg *et al*. 2014) [16].

In CRISPR/Cas9 innovation, the Cas9 and sgRNA are communicated and structure or build a perplexing that wires on focused DNA close to the NGG (PAM) site. A twofold stranded break (DSB) is actuated at a focused on location that can be fixed either by non-homologous end joining (NHEJ) or homology coordinated fix (HDR). The fix by NHEJ as a rule brings about the addition or erasure (indel) or edge move change coming about quality knockout by interruption. On the off chance that a giver DNA is given end homology, at that point this can be embedded at the focused in the vicinity to adjust a quality by including changes in nucleotides or by quality inclusion (Cong *et al*. 2013) [4]. In type II of CRISPR, attacking viral DNA or plasmids is divided into smaller pieces and integrated in CRISPR locus. The particular loci are reproduced or transcribed and these processed transcripts produce crRNA. These crRNAs regulate effect or endonuclease to targ*et al*ien DNA depending upon complementarity or matching of sequence.

Spectacularly, the sgRNA programmed Cas9 appeared more effective in targeted gene modifications rather than individual trRNA and crRNA. Till date, genome-editing protocols have ratify three different types of Cas9 nuclease. The first Cas9 type can cut DNA site-specifically and results in the stimulation of DSB repair event to occur. Cellular NHEJ (Non-Homologous End Joining) mechanism is used to repair DSBs (Sternberg *et al*. 2014) [16]. As a culmination, insertions/deletions (indels) takes place that obstruct the targeted loci immediately. Apart from this, if any similarity between donor template and target locus is witnessed, the DSB may be repaired by HDR pathway (homology directed repair) allowing exact substitute mutations to be prepared (Sternberg *et al*. 2014) [16].

CRISPR/Cas9 technology based resistance in plants

The CRISPR/Cas9 framework is a recently created instrument that can outfit significant job in creating opposition in various agrarian yields. The CRISPR intervened genome altering has been utilized as a device for bestowing protection from infections in plants made out of Cas9 endonuclease of Streptococcus pyogenes and an engineered guide RNA (gRNA), which consolidates elements of CRISPR RNA (cRNA) and trans-initiating cRNA (tracrRNA) to guide the Cas9 protein to the DNA target arrangement driving the protospacer-related theme (PAM) (NGG on account of *S. pyogenes*). Since the particularity of the framework is dictated by the 20-nucleotide arrangement of the gRNA, it takes into consideration impossible to miss and satisfactory genome.

The role of CRISPR/Cas9 system for developing resistance in plants

1. Antiviral Resistance in Plants Based on CRISPR-Cas Technology

The CRISPR/Cas arrangement of genome altering has now been utilized proficiently as an instrument for giving protection from infections in a few harvest plants (Chaparro-Garcia *et al*. 2015) [3]. In all respects as of late, three reports have been recorded which depicted the CRISPR/Cas approach and its wide utility for security to plants against geminiviruses (Ji *et al*. 2015) [6]. This innovation conceded improved protection from the plants against the geminiviruses species including BCTV (Beet wavy top infection), TYLCV (Tomato yellow leaf twist infection), and MeMV (Merremia mosaic infection). The CRISPR–Cas framework at first perceive and focuses on the particular site of the hereditary material of attacking pathogens by means of; three stepwise forms, to be specific acquisition, expression, and impedance. The procurement step is the principal or beginning occasion which includes acknowledgment and joining of remote DNA as spacer at the pioneer side of the CRISPR locus, trailed by duplication of the rehash. When all is said in done, in initial step, short part of exogenous DNA are fused by means of CRISPR cluster in the bacterial genome, and goes about as another spacer arrangement. In the articulation venture of the CRISPR–Cas framework, the long pre-CRISPR RNA (pre-crRNA) first transcribed and is then actively processed into crRNAs with the help of specific Cas proteins as well as trans-activating crRNA (tracrRNA). During the third or final event as interference, a specific sequence of foreign genomic element is targeted and then cleaved into small fragments. The crRNA directs the Cas9 protein for cleavage to the complementary target region of the DNA of viruses and plasmids and therby leading induction of resistance in plants (Kumar and Jain, 2014) [9].

2. CRISPR/Cas confers resistance in plants against fungal and bacterial pathogens

Fungal pathogens are the main factors responsible for the most severe diseases affecting plants, leading to significant reduction in yield and crop quality and causing enormous economic losses worldwide. It is estimated that around 30% of the emerging diseases are caused by fungi (Giraud *et al*., 2010**)** [5] despite of large applications of CRISPR/Cas9 in managing plant virus disease, recently it has also proven to be extremely versatile tool for combating fungal and bacterial pathogens. Rice-pathogenic bacteria *Burkholderia glumae*, *Burkholderia gladioli*, and *Burkholderia plantarii*, which primarily cause grain rot, sheath rot, and seedling blight, respectively, can severely affects and reduce rice yield potential in all the major rice growing countries. For successful managaemnt of these diseases yet requires comprehensive studies and new tool to control these diseases only in the early stages. Biological control approach, frequently referred to the use of non-pathogenic microbial antagonists or products derived from their

metabolism, represents a valid and promising alternative under a more ecological perspective to reduce the activities and to control populations of target pathogens (Singh, 2016**)** [14]**.** Although the genome of *B. plantarii* ATCC 43733T has many related features with those of *B. glumae* and *B. gladioli*, but this *B. plantarii* strain also has some distinctive features, including quorum sensing and CRISPR/CRISPR-associated protein (Cas) systems, signifying that *B. glumae* has evolved rapidly or has undergone rapid genome rearrangements or deletions in response to the host plants. Thus, this rice pathogenic Burkholderia species has unique features relative to other *Burkholderia* species of plants, animals and humans (Seo *et al*. 2015). The type II bacterial CRISPR/Cas9 system has been used to efficiently disrupt target genes in the smut causing maize pathogen *Ustilago maydis*. Two years ago TALEN and CRISPR/Cas9 technologies were used successfully to target the mildew-resistance locus O (MLO) in wheat, generating plants resistant to powdery mildew disease (Wang *et al*. 2014) [7]. GETs were used to generate plants resistant to bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *oryzae*, impairing down the transcriptional regulation of S-genes by the effector and thereby leads to resistance process. Indeed, the plants firmly edited in the *OsSWEET14* promoter were resistant to bacterial strains since the effector was found incapable to activate the transcription of its target (Li *et al*. 2013) [10]. The metabolic pathways that regulate hormonal balance can be customized to enhance the IMC component of plant immunity. This goal was achieved by using GETs to cause the down-regulation of ethylene-responsive factors (*ERF*). In particular, the ethylene pathway in rice was successfully modified by targeting a mutation in OsERF922 using CRISPR/Cas9 technology to increase resistance to rice blast pathogen caused by *Magnaporthe oryzae* (Wang *et al*. 2016) [6].

The CRISPR/Cas9 also holds potential for generating mlo-type resistance in tomato against powdery mildew and other pathogens. *Pectobacterium atrosepticum*, a plant pathogen that causes soft-rot and blackleg disease in potato, has been used to explore protein-protein interactions and complex formation in the subtype I-F CRISPR/ Cas system including Cas1, Cas3, and the four subtype specific proteins Csy1, Csy2, Csy3 and Cas6f (Richter *et al*. 2012) [13]. The CRISPR/Cas9 system and a synthetic sgRNA targeting the CsPDS gene were delivered into sweet orange leaves via agroinfiltration facilitated by pretreatment with *Xanthomonas citri* subsp. *citri* (Xcc) DNA sequencing confirmed that the CsPDS gene was mutated at the target site with efficiency of approximately 3.2-3.9 % and without any off-target mutagenesis, suggesting targeted genome modification in citrus using the Cas9/sgRNA system-a system holds considerable promise for the study of citrus gene function and for targeted genetic modification (Jia and Wang, 2014) [7].

Advantages of CRISPR/Cas 9 technology

The ease, simplicity, a relatively high degree of precision and accessibility of the CRISPR/Cas9 as a new gene editing technology platform offers substantial improvement and advantages over other genome editing methods, on a genomic scale. The main advantages of genome engineering using the CRISPR-Cas9 system are:

1. **Site specific mutagenesis:** The CRISPR/Cas 9 is a very versatile and comparatively precise approach to carry out strand-specific cleavage and modification of DNA at specific sites.
2. **Minimizing off-target mutations:** To minimize off-target activity, a double nicking strategy can be employed to introduce DSBs at the target site of the genome.
3. **Applicability across a wide range of organisms with multifunction:** CRISPR/Cas has already been discussed in many species with 33–92% success rate, nutritional values or stress and crop improvement in which genome engineering has been difficult.
4. **Multiplexing:** The main practical advantage of CRISPR/Cas is that it can simultaneously introduce multiple gene disruptions, which allows researchers to edit multiple genes in one plant line through a single transformation without time-consuming which is otherwise difficult to achieve through other gene editing technologies.
5. **Efficient and easy to use:** CRISPR/Cas9 technique is affordable, easy to use, and importantly it works for high throughput multi-gene experiments.
6. **Inexpansive and less time consuming:** Older genome editing tools, such as ZFNs and TALENs, are slow and expensive due to their use of proteins necessary for findng out the portion of DNA to be cut.

Future aspects

The CRISPR/Cas9 innovation presents inventive atomic scissors for designing science and has as of late turned out to be one the most adaptable and prevailing stages. One of the most significant part of this innovation is to permit absolutely and unsurprising various hereditary changes all the while. Despite the fact that the CRISPR/Cas9 framework is a magnificent apparatus for genome altering, however the degree of off-target change should be investigated with high cleavage effectiveness among various yet flawlessly coordinated targets. Be that as it may, improvement in this method could be conveyed in the forthcoming future to battle dangerous phytopathogenic infections. Toward this path research works by different farming researchers are in advancement and ideally, we will get the most ideal outcomes soon.

Conclusion

The CRISPR/Cas framework is an amazing promising device for genome altering in plants because of its effortlessness, proficiency, insignificant off-target impacts, and high explicitness for focused mutagenesis. It gives a novel stage to opposition actuation through continous rearing in yields. Critically this procedure has now been advanced as head system that has made it conceivable to adjust have genome by presenting some real changes. These alterations incorporate quality substitution, cancellations, reversal, knockouts, and translocations. The most potential prospects of this system for delivering plants with transformations likewise contribute incredibly to different controls of science, i.e., engineered science, biofuel creation, ailment obstruction, abiotic stress resistance, phytoremediation and so on. Without a doubt, CRISPR/Cas9 framework can encourage intense research on genome adjustments in yield plants yet enhancements in grouping particularity and decrease of askew impacts must be redressed, perspectives should have been considered for the structure of the gRNA-Cas9 complex. Through this

innovation latent obstruction could be misused with the guide of the CRISPR/Cas9 framework to make novel opposition alleles in yield plants to ensure them against tricky infections utilizing host interpretation inception factors. Another noteworthy property of this CRISPR/cas9 framework is that it is by a wide margin the most easy to understand framework among all the right now accessible genome altering methods. With this incredible and creative strategy, opposition in plants could be propelled which accordingly contribute for reasonable farming later on for boosting yield by battling abiotic and biotic burdens.

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