*Biological and Clinical Sciences Research Journal*
*ISSN: 2708-2261*
*www.bcsrj.com*
DOI: https://doi.org/10.54112/bcsrj.v2022i1.97
*Biol. Clin. Sci. Res. J., Volume,* ***2022****: 97*
***Review Article***

# ROLE OF CRISPR TO IMPROVE STRESS TOLERANCE IN PLANTS

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*(Received, 13th January 2022, Revised 28th August 2022, Published 6th September 2022)*

**Abstract:** *Climate changes and increasing human population is experiencing by most of the countries throughout the world, so, for production of crops with enhanced adaptation to the environment and high yield reliance through conventional breeding technologies seemed to be fully supporting now a days. It requires those techniques that increase crop yield in less time through developing resistance of plants for stress factors. Fortunately, for improvement of crops under the abiotic and biotic stress conditions, clustered regularly interspaced short palindromic repeat (CRISPR) approach provided a way towards new horizon and consequently revolutionizing the plant breeding approach. This review article presents the optimization and mechanism of CRISPR strategy and its huge number of applications for crop improvement like domestication, fruit quality improvement, resistance to abiotic and biotic stresses is most highlighted aspect. In this review article there is a brief summary about CRISPR/Cas9 technique and its role in increasing agricultural yield by gene knock in or knock out. It also presents number of evidence based studies where this approach has been used for making plants resistant to biotic factors. Future perspectives and controversies have also been discussed.*

**Keywords:** biotic stresses, crop yield, conventional breeding, CRISPR/Cas9 system, gene editing

## Introduction

The environmental condition by which growth, productivity and development of plants is affected is called as stress in plants. When a plant undergoes stress condition, number of altered mechanisms and processes are activated by plants like crop yield, variations in rate of growth, cell metabolism and altered gene expression. When some changes occur in environmental conditions, plant stress takes place. But those plants that are stress tolerant, when exposed to stress conditions, they become able to resist that stress in a time dependent manner. There are two types of plant stresses: biotic and abiotic stress. Physical and chemical stress exposed on plants by the environment is known as abiotic stress, while stress on plants due to insects, microbes, or some other biological units is known as biotic stress (Verma *et al*., 2013). Number of adverse environmental conditions and biotic stresses are faced by plants in terms of molecular, biochemical and morphological mechanisms (Bostock *et al*., 2014). Biotic and abiotic stresses cause a major loss of plant and crop productivity like literature studies suggested that 28.8% loss of cotton, 27% loss of soybeans, 41% loss of potatoes, 31.2% loss of maize, 37.4% loss of rice, and 28.2% yield loss of wheat occur every year. Seed damage, chlorosis, leaf wilting, necrosis, patches on plants, leaf spots and root rot, etc. are the symptoms caused by plant stresses (Wang *et al*., 2013; Bhatta and Malla, 2020). Global warming, melting of ice, rising of sea levels are the risks for agricultural production. Many problems of plant stresses may be controlled by adopting different methods that can help in recovering the soil and plant properties. Chemical and biological alterations are the most effective implements in enhancing various soil properties of sodic soils (salines), it also endure the food production and authenticates inorganic and organics amendments to put back soil quality. For the increasing population, low crop productivity and decreasing soil fertility has raised the interest of researchers and scientists (Kasim *et al*., 2018; Wang *et al*., 2016).

Stresses may results in plant injuries by several metabolic dysfunctions. If stress is short term or mild plant may recover from it, but if stress is severe plant death occur due to blockage of induced senescence, seed formation and flowering, such plants are called as stress susceptible. But there are some plants like (Ephemerals) desert plant that is able to escape from

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stress condition (Zhu, 2002). When plant undergo stress its ability to take up the nutrients is reduced which results in plants death. Due to post harvest and pre harvest losses biotic stresses now has become a major issue (Gull *et al*., 2019). Plants may evolve certain sophisticated strategies to counteract the biotic stress conditions. Defense mechanisms of plants that act against such stress conditions are controlled genetically by the plant genome. There are hundreds of genes encoded by plants that act against biotic stresses (Gull *et al*., 2019). Abiotic stress is different from biotic stress which is imposed on plants by non- living factors like drought, floods, cold, temperature, sunlight, and salinity having negative influence on plants. Which type of stress to be imposed on plant is decided by the climate in which plant is growing and ability of plant to tolerate that stress condition? Photosynthesis is affected by biotic and abiotic stresses, for example leaf area is reduced by chewing insects and rate of photosynthesis per leaf area is reduced by virus infection (Sah *et al*., 2016; Sergeant and Renaut, 2010). Even though abiotic stresses like drought and heat take over the entire diversity of plants, biotic stresses can also be absolutely vital on a variety of geographical region. Furthermore, unlike abiotic stresses, the contact between the two living things is added to the investigation of the relationship among plants and pathogenic organisms. The damage and destruction caused by herbivorous insects, nematodes, bacteria, fungi and others to crops and the communities that rely on these crop production is well known, and it was one of the causes of the large scale immigration of Irish agricultural producers to North America in the middle of the 19$^{th}$ century after potato crop attracted by *Phytophtora infestans* that results in the movement of or displacing 25% of the Irish population from their houses (Fraser, 2012).

Fungicides and pesticides are mostly used for controlling the reduction in yield due to do biological factors, but they are very harmful for human health environment, and aquatic life etc. Now a big concern is that those areas which are good for genetically uniform crop may be devastated by the spread of disease due to biological factors like *Fusarium oxysporum* species (O'Donnell *et al*., 1998). Understanding and controlling the interconnections between pathogens and plants requires unravelling the processes underlying in resistance and disease (Göhre and Robatzek, 2008). From seed emergence to the development of entire plant, they face interactions with pathogens or microbes during entire life cycle, but, some plant evolve natural mechanism and pathways to counter harmful pathogens and microbes. Different chemical and physical barriers are developed by plants to control the plant interaction with dangerous or growth retarding microbes (de Wit, 2007). Plants will engage with pathogens and microbes throughout their lives, from emergence of seed to plant production, and their longevity will rely on the processes they have developed to combat pathogenic organisms. Plants have a variety of chemical and physical barriers that hinder nearly all unfavorable plant biotic stressor interrelations from forming. For the constitutive defense like storage of secondary metabolites with properties or deterrent properties chemical components of plants are very important that can inhibit or act as toxic compounds for enzymatic functions of disease causing microbes. Although all plants build physical boundaries, such as cell walls and cuticle, to prevent unpleasant biotic interactions, the amplitude and form based on the species and environment. However, the adaptability of such survival mechanisms to changing circumstances is slow. Plants have adapted a method that enables for rapid and directed responses in order to react to pathogen attack (Dangl and Jones, 2001).

**CRISPR/Cas9**

Currently the population of world is 8 billion which has been predicted to be increased to 9 billion by 2030, 10 in 2060 and 11 in 2090 according to World Health Organization (World Health Organization, 2019). Food demand has increased and keeps rising as the world's population rises, so as a consequence, agricultural output must stay consistent with the continuously increasing supply. But in recent years, challenges for the agricultural production has also been increased like increase of pests, insect diseases, abiotic stresses like drought, floods, salinity, temperature *etc*. (Ort *et al*., 2015; Velásquez *et al*., 2018). Establishing high-yielding crop production which are immune to abiotic and biotic stress is one way to solve rising agricultural stresses and meet rising food, fuel and feed demand. Previously, traditional breeding and transgenic plants have been effective methods for introducing essential genetic changes for genetic improvement (Schaart *et al*., 2016). Moreover, conventional breeding for improvement of plants if used require long time, more space and expensive method (Abdelrahman *et al*., 2018). The GM (genetically modified) crops have the capacity to resolve the short comings of conventional breeding, but they are fraught with complications. Such plants are subject to stringent rules and regulations in terms of export, import and utilization. Finally, the possible effect of genetically modified foods on global food production is determined by how well the public and governing bodies perceive the technologies (Voytas and Gao,

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2014). Now a day there are large number of people who are not in support of using genetically modified crops, similarly some international markets are not supporting and accepting such foods. In the field of agriculture, site-directed nucleases (SDN) which help in genome editing tools have provided a new way for genome editing. There are many benefits of genome editing but by this method no foreign is inserted and have benefits for regenerative medicine, therapeutics and opportunity in improving the livestock, disease resistance of crops and traits of plants. So, it is the recent and widely used techniques for genome editing of plants is CRISPR/Cas9 (Jinek *et al*., 2012).

Prokaryotic organisms have naturally evolved defense mechanisms against their harmful predators and phage predation. CRISPR is providing heritable and adaptive immunity against pathogens and viruses. It is composed of 20-50bp sequence which is separated by spacer sequence of similar length. CRISPR associated (Cas9) protein is the main protein which is linked with this process. CRISPR-Cas9 is very effective and efficient process that helps the plants to combat abiotic and biotic factors and leads to increase the productivity of plants. Genome editing mediated by CRISPR-Cas help the plant researchers in achieving desired traits under in vivo, ex vivo and in vitro conditions. Various tools along with CRISPR-Cas system help in transcriptional repression, activation, prime editing, base editing, epigenome editing, multiplex editing, and targeted modifications in genome. To the template DNA Cas9 nuclease binds and cut at the desired or targeted site by the help of CRISPR RNA (crRNA) which is a short RNA molecule. This short RNA molecule sometimes fuses to form (sgRNA) which is a single guide RNA. A DNA endonuclease known as Cas9 composed of 1368-amino-acids that incise DNA by its two nucleases domain at 3 bp sequence upstream of PAM. Cas9 has RuvClike nuclease domain which helps in cleaving non target sequence that are complementary to DNA sequence, and HNH-like nuclease domain that cleave the target sequence complementary to sgRNA. Spacer acquisition and crRNA maturation are achieved by Cas9. Protospacer adjacent motif (PAM) is a short sequence which flanks the target site help in the recognition of targeted sequence by base pairing. Between target DNA and single guide RNA, complementary base pairing take place simultaneously and leads to strand incision and R-loop formation (Gurumurthy *et al*., 2016; Zeilmaker *et al*., 2015). After that various conformational changes and Cas9–DNA interactions take place. Damaged DNA then undergo following repair pathway: homology directed repair (HDR) the high-fidelity pathway and non-homologous end joining (NHEJ) which is an error-prone pathway.

There are three categories of CRISPR-Cas system: Type I, II, III. Archaea and bacteria have type I CRISPR-Cas mechanism on the base of specific signature of Cas protein. It can bind to DNA sequence by the help of Cas3 protein endonuclease activity. In bacteria Type II system has been found and it is very simple with Cas4/Csn2, Cas2 and Cas1 proteins along with Cas9. In archaea Type III CRISPR-Cas system has been discovered which target both DNA and RNA. Through the help of Cas6 and Cas10 it can be recognized. Targeted DNA is cleaved by the help of Cas10 protein. This system is also present in bacteria negatively regulating genes can he targeted in *Streptococcus pyogenes* by SpCas9 of Type II CRISPR system (Mao *et al*., 2013; Miller ad Cross, 2011). First gene targeting by this system was achieved in *Nicotiana tabacum* (tobacco). In earlier times genome editing has been done by the help of Transcription Activator-like Effector Nucleases (TALENs) and Zinc-Finger Nucleases (ZFNs) hybrid protein. In ZFNs with the base pair amino acid interaction is complex. Immunity system by CRISPR/Cas has been divided into 3 phases: in locus new spacers are inserted, the Cas gene expression and finally precursor CRISPR RNA transcription (Wang *et al*., 2016; Zaidi *et al*., 2016). It will leads to pre-crRNA maturation with the help of Cas protein. Then in final stage degradation at the targeted site achieved by the help of Cas protein and crRNA activity.

**CRISPR importance for plants and animal**

This process had been first published in the 1980s, it was only a decade earlier that its true power hasrealized. During this brief period, there was considerable debate and interest about its use in plant, animal and human applications. The strategy is used in both reverse and forward genetics (Gurumurthy *et al*., 2016). In humans, there has been a surge of interest in handling diseases due to age like colon cancer and Huntington's disease, as well as inherited genetic diseases such as sickle cell anemia (Ye *et al*., 2016). Numerous types of animal research have been completed, like developing avian leucosis virus resistance in chicken and increasing body mass in goats (Koslová *et al*., 2018; Miao *et al*., 2013). CRISPR/Cas9 has been widely used in agricultural system for improving plant resistance to biotic and abiotic diseases by overexpressing resistance genes and knocking out susceptibility genes. Powdery mildew resistant grapes, powdery mildew resistant apple, canker resistant citrus, blast-resistant rice (Wang *et al*., 2016) powdery mildew resistant wheat andcucumber vein yellowing virus-resistant wheat

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are the examples of some crops that have been improved by CRISPR/Case System (Chandrasekaran *et al*., 2016). But like any other technologies that have been recently innovated, it is also under controversies because of gene-editing of a human embryo by different researchers (Cyranoski and Ledford, 2018).

**Reaching the CRISPR Age**

Genome or gene editing is defined as variations in the deoxyribonucleic acid (DNA), of an organism by modifying, replacing or adding the genetic material. SDN through CRISPR/Cas9, ZFNs and TALENs have been used to edit the genome (Čermák *et al*., 2017).There are number of gene editing tools like CRISPR/Cas9, TALENs, and ZFNs, mega nucleases that are dependent on the artificially engineered SDN and they used for introducing mutations by DNA modification in number of agriculturally important plants. Modification of DNA can be done in the form of substitutions, insertions, deletions or single-nucleotide polymorphism (SNPs). All of such genome editing techniques depends on DSBs that can be repaired by cell's repair mechanism (Vats *et al*., 2019). This gene-editing technology now has undergone a huge number of transformations and developments. For example, genetic transformation help in the replacement of the yeast chromosomal segment that is artificially engineered (Bhatta and Malla, 2020). Similarly, linking of endonuclease FokI with zinc finger proteins enables DNA cutting at predetermined sites. Moreover, for producing DSBs at targeted and specific sites in TALENs technique is very well knownediting tool in crops. Different researches have applied such gene editing tools like TALENs in Arabidopsis, tomato (*Solanum lycopersicum*), maize, wheat, rice (*Oryza sativa)*, ZFNs in soybean (*Glycine max*) (Bhatta and Malla, 2020). But, TALENs and ZFNs needs complex tools by which plant applications are limited (Mishra *et al*., 2013).Researchers are continuing their search forcreating a gene-editing method to overcome such challenges because of intensive protein engineering requirements. CRISPR/Cas9 has been developed in archaea and bacteria as an adaptive immune response to plasmid and viral DNA (Doudna and Charpentier, 2014).

**Applications of CRISPR/Cas9**

CRISPR/Cas9 is very popular from all the genome editing techniques that are available, in agricultural system. Different gene editing tools have been evolving at rapid rate, but CRISPR/Cas9 is still routinely used, precise and an efficient gene-editing platform. Higher efficiency has been found in plants that are edited by gene editing technique. For example, 79% in maize and 91.6% in rice (Miao *et al*., 2013). This technique has the potato to behave as an effective plant breeding approach thathas been widely used for editing the plant genome. It allows multiplexing to edit multiple loci frequently and can introduce multiple double stranded breaks and it has a very simple design (Bhowmik *et al*., 2018; Mao *et al*., 2013). A number of crops have been edited by using this technique and so used for understanding how plants tolerate biotic and abiotic stress factors. It acts as a foundation for breeding program by the identification of different genes that can contribute to a particular trait. It will enable introgression and crossing strategies. Into elite germplasm, novel mutations can be introduced to accelerate the breeding program.

**Role of CRISPR for plants under biotic stress**

Thus far, the CRISPR/Cas9 genome engineering scheme has been used in about twenty horticultural crops for a diverse array of characteristics including biotic and abiotic stress control and yield enhancement (Ricroch *et al*., 2017; Sentmanat *et al*., 2018). Many of the research papers are referred to as strong evidence studies because they describe the use of the CRISPR/Cas9 strategy to remove specific genes associated in abiotic and biotic stress resistance mechanisms. Different microorganisms are involved in stress in plants and cause number of challenges in developing disease resistant plants that account for more than 60% of potential yield reductions and 70-75 percent of global food output reductions. CRISPR/Cas9-based genetic changes have also enhanced crop stress tolerance and invulnerability to massive conditions such as drought. The section gives an overview and application of CRISPR for genetic manipulation in a variety of horticultural crops (Ren *et al*., 2016; Vella *et al*., 2017). CRISPR-Cas9 is emerging as promising method for genetic engineering improving favorable crop traits such as yield, plant architecture resistance to disease, nutritional composition, and stress adaptation. Knockout of disease causing genes can enhance a specific trait in certain cases. Rice grain weight increased after some QTL genes were modified (Xu *et al*., 2016). ARGOS8 locus has been genetically altered in maize and it resulted in increased yield of maize crop (Wang *et al*., 2018). In the first transgenic generation CRISPR-Cas9 produced mutants in woody plants, it was significant in such plants because of their long lifespan (Borca *et al*., 2018; Fan *et al*., 2015). In rice, OsGAN1 gene has been knocked out which results in regulation of plant height and length (Ma *et al*., 2016). Similarly, OsTCD10 has been knocked out that had role in cold stress resistance and knock out of OsABCG26 gene regulate the anther cuticle and pollen exine (Chang *et*

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*al*., 2016). CRISPR-Cas9 has been used efficiently for genome editing of different crops like wheat, rice, maize and cotton. Most of the genome editing techniques targeted those genes that are susceptible to biotic stress factors. CRISPR-Cas9 method in wheat has been used for knocking out the EDR1 homologs to create plants (Taedr1) having higher tolerance to powdery mildew disease (Zhang *et al*., 2017). Knockout of susceptible gene EDR1 in Arabidopsis resulted in increase of plant resistance to powdery mildew. In several dissimilar hosts, eIF (eukaryotic translation initiation factor), and recessive resistant genes have been detected with eIF4E and eIF (iso) 4E genes used with CRISPR-Cas9 to form a plant that is resistant to viruses in cucumber and Arabidopsis (Datsenko *et al*., 2012; Pyott *et al*., 2016).

CRISPR-Cas9 was used to edit CsLOB1 that is susceptible to citrus canker caused by *Xanthomonas citri*. Resistant grapefruit plants were produced by CRISPR-Cas9 gene editing tool (Jia *et al*., 2017). MLO gene that is susceptible to powdery mildew disease was knocked out by CRISPR-Cas9 and it enhanced resistance to powdery mildew in wheat and tomato (Humphry *et al*., 2006; Pan *et al*., 2016).The viral infection that caused cleaved beet severe curly top virus in beet has been decreased by CRISPR-Cas9 (Macovei *et al*., 2018). The eIF4G gene, was linked to rice tungro spherical virus (RTSV) and this gene has been knocked out by this approach for developing resistant rice varieties (Macovei *et al*., 2018). VvWRKY52 gene has be knocked out for developing grapes resistant to Botrytis cinerea by CRISPR-Cas9 (Wang *et al*., 2018). TYLCV, TYLCSV and CLCuK0V have been interrupted by CRISPR-Cas9. So, to RNA viruses' molecular immunity can be mediated by expression of /FnCas9 in Arabidopsis and tobacco (Zhang *et al*., 2018). In rice OsERF922 has been targeted against blast fungus by CRISPR-Cas9 (Peng *et al*., 2017; Wang *et al*., 2016). Tolerance against various stresses can be controlled by Plant ethylene-responsive factors (ERFs) as they are involved in the ethylene (cytokinin) pathway (Jung *et al*., 2007; Ueta *et al*., 2017).

Generally, the CRISPR-Cas9 system's aims to invading or occupy DNA which is divided into phases (Amitai and Sorek, 2016). From target DNA, spacer sequences are derived after the identification of invading DNA and then inserted into the CRISPR system of host for establishing immunological memory, known as acquisition stage. Second stage is expression stage in which Cas9 protein is expressed by transcribing into pre-crRNA. CRISPR RNA (crRNA) which is a non coding trans-activating RNA hybridized to Cas9 and pre-crRNA protein which is processed to crRNAs that are mature RNA units. Third is interference stage in which Cas9 protein recognizes the target DNA by the help of crRNA which leads to degrade or cleave invading foreign DNA. DNA is cut into pieces by Cas9 protein which produces DSB and activates the repair mechanisms of DNA. When homologous repair template is absent, NHEJ pathway is activated that introduce deletion, substitution or insertions randomly at the double stranded break sites which results in gene function disruption. If DSB site that surrounds the DNA have homologous sequence, HDR pathway is initiated which results in mutations that help in precise genetic mutations like mutations, deletion, and knock in or out (Fister *et al*., 2018; Malnoy *et al*., 2016; Yin *et al*., 2016). For exploiting this strategy for the editing the genome, sgRNAs are needed for constructing CRISPR-Cas9 expression cassettes. To specific genomic sites, Cas9 protein is then guided by the sgRNAs which identify NGG-type protospacer that is adjacent motif which targets DNA sequences by Watson–Crick base pairing (Liu *et al*., 2017; Tang *et al*., 2017).

**CRISPR-Cas9 system Optimization**

In 2013, CRISPR-Cas system was engineered for editing the genome of plants but number of efforts has been made for transforming it into an efficient tool. In present era, this technique has multiplex editing capability which is able to edit multiple genes at one time. Additionally, this system can target ncRNAs including microRNA42 and long ncRNA41, untranslated region of one coding gene and open reading frame (ORF), promoter regions. At genomic targets single base substitutions without requiring DSBs can be obtained. There is a description of optimization of the CRISPR-Cas system for empowerment of sgRNAs with multiplexing capability, the optimization of Cas9 promoters and diversified development of Cas9 proteins (Chen *et al*., 2017; Wu *et al*., 2017). An edible, perennial fruit crop has been defined by DuaneGreen thathas been obtained by this technique and of economic importance. Melons, cucumbers and tomatoes are grown as annuals fruit crops. Tomato is a model plant of fruit biology due to ease of genetic transformation, short growing period, efficient breeding, simple diploid inheritance and easily achieved germplasm resources from an extensive research. There are number of applications has been described here (Miller and Gross, 2011). Tomato is the first genetically engineered crop produced by CRISPR-Cas9 system in 2014. Wiry phenotypes have been produced by ARGONAUTE7 promoter which was knocked out, laminae lacking leaves has been formed and leaflets without petioles. Number of research

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articles has been published on applications of tomato by CRISPR-Cas9 system. They have classified those applications into following groups: domestication of tomato, improvement of tomato fruit quality, resistance to abiotic stresses and resistance to biotic stresses (Borca *et al*., 2018; Brooks *et al*., 2014).

**Resistance to biotic stresses**

Insects, fungi, bacteria and viruses are all biological factors or stressors which cause harmful effects in plants (Langner *et al*., 2018). Since 2013 CRISPR-Cas9 approach has been used for solving agricultural issues like to control biological stresses. So, from that time scientists and researchers started to control viruses, fungi, bacteria and other biological stressors by genome editing to make plants resistant or fight for such factors (Arora and Narula, 2017). Two different approaches have been used for combating viruses: first alter the genes that confer antibacterial, antifungal or antiviral properties and second to directly target the genome of biological factors by creating sgRNAs. CRISPR-Cas9 system was used by Tashkandi *et al*., (2018) to create resistant plants of tomato to yellow leaf curl virus that target replicase loci and genome's coat protein. Such transgenic plants found to give higher yield by accumulating less amount of viral DNA or showed effective viral combating ability as compared to wild plants. Research also claimed that this resistant ability of tomato plants persisted for many generations (Tashkandi *et al*., 2018). This technology can also be used in knocking out those genes that are involved in resistant pathways for creating or generating immunity in plants against biological stress factors. Wang and coworkers targeted Tomato Dicer-like 2 (DCL2) genes and found that this gene exhibit viral (Wang *et al*., 2018; Zhang *et al*., 2018).

Rot, rust, smut, mildew are some common diseases caused by fungi that results in reducing agricultural yield and significant crop loss. In tomato powdery mildews and downy caused by fungal strains. A member of the 2-oxoglutarate Fe(II)-dependent oxygenase superfamily known as DMR6 (Downy mildew resistant 6 ) involve bin homeostasis of salicylic acid in Arabidopsis thaliana. Its overexpression raises the susceptible of plant to mildew disease. DMR6 ortholog was inactivated by using CRISPR-Cas9 system in tomato by some researchers and found that mutants showed disease resistance against different biological factors like *Xanthomonas* spp., *Phytophthora capsica*, and *Pseudomonas syringae*. Susceptibility to fungal strain that cause powdery mildew disease is due to membrane associated protein which is encoded by Mildew resistant locus O 1 (Mlo1). Nekrasov and coworkers used CRISPR-Cas9 system for developing tomato mutants in which Mlo1 locus is missing. They suggested that mutants were completely resistant to mildew *Oidium neolycopersici* fungus (Nekrasov *et al*., 2017). The callose synthase is an enzyme that is encoded by Powdery mildew resistance 4 (PMR4), which is linked with it's ability to resist *O. neolycopersici*. Fusarium wilt disease is caused by *Fusarium oxysporum* in tomato, which results in reduced yield. Solyc08g075770 is the gene that is linked with the susceptibility of tomato to *Fusarium* will. Researchers knocked out this gene with the help of CRISP/Case System and made *Fusarium* resistant tomatoes (Prihatna *et al*., 2018). an airborne plant pathogen known as *Botrytis cinerea* which leads to grey mould disease in tomatoes. Yu and his coworkers created *B. cinerea* resistant tomatoes with the help of CRISPR/Case Systemby knocking out mitogen-activated protein kinase 3 (MAPK3) (Yu *et al*., 2018). Genetically resistant plants are the most effective way to cope up with environmental and biological stress factors because it very difficult to main environmental and biological factors due to poor agricultural system (Borrelli *et al*., 2018). In tomato *Pseudomonas syringae* caused bacterial speck disease by which marketability and productivity of tomato has been reduced. Jasmonate zim domain protein 2 (JAZ2) is responsible for susceptibility of tomato which was reduced by using CRISPR-Cas9 system (Gimenez-Ibanez *et al*., 2017).

**CONCLUSION**

Number of advanced plant breeding techniques provides the researchers ability to quickly and precisely insert required genes as compared to conventional breeding techniques. A fundamental breakthrough technique is based on CRISPR/Cas9 approach. Prominent areas of work in the future of CRISPR/Cas9 system are disease resistance, nutritional value, enhance yield. Since last five years, this system has been applied in different plant systems for combating abiotic, biotic stresses and functional studies for improving agronomic traits. By different modifications in this approach, on-target efficiency can be increased and most of the work require further improvement and preliminary needs. CRISPR/Cas9 based genome editing in future will gain more attention and be an important technique for obtaining suitably edited' plants that may help in maintaining the growing human population and achieving the zero hunger goal. The establishment of an evaluation system and further advances in CRISPR technology is necessary. Number of counties must be coordinated for fostering inclusive and optimistic attitude toward CRISPR-edited crops. For ensuring maximum benefit by minimizing risks, this technique public and market acceptance by

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proper guidelines. Basics of this technique must be explained to public and this may increase its acceptance, but researchers should follow all the ethics while performing experiments.

**Conflict of interest**

The authors declared absence of conflict of interest.

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